



WPC2016

THE XXV WORLD'S POULTRY CONGRESS

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The Proceedings of XXV World's Poultry Congress 2016 — Abstracts



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The Proceedings of XXV World's Poultry Congress 2016 —— Abstracts

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Xiangping Liu and Changxin Wu

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S1- 0002 Male broiler performance responses to different levels of l - carnitine from 11 to 45 days of age

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The present study was conducted to determine the effects of different levels of L- Carnitine (Lohman) on three hundred and sixty 11 days-old (270 ± 4 g) male broiler chicks (Ross 308) in a completely randomized experimental design with 6 treatments (L-Carnitine 0, 75, 150, 225, 300, 375 mg/kg) and 6 replicates in 36 pens. The highest body weight (2852.8 g) was found at the level of 225 mg/kg with beneficial effect on feed intake and lowest level in feed conversion ratio ($P < 0.05$). The periods of 39-45, 25-45 and 11-45 days of age, highest feed intake observed in the control group without L- Carnitine and the lowest feed intake observed in broiler fed diet at 300 mg/kg ($P < 0.05$). The periods of 25-45 and 11-45 days of age, highest feed conversion ratio observed in the control group without L-Carnitine that was 2.24 and 2.2; and the lowest FCR observed in treatment 225 mg/kg that was 2.05 and 2.03, respectively ($P < 0.05$). It seems that the effects of L-Carnitine on performance are associated with the age of the bird. Supplementation of L-Carnitine can improve energy efficiency. And in the end it can be resulted that supplementation of L-Carnitine in male broilers diets with a positive impact on weight gain and decreasing feed consumption improves feed conversion ratio in growth period and final period. In conclusion, we suggested that the best level of L- Carnitine was between 150-300 mg/kg for optimum economical production performance in male broiler duration from 25 to 45 days of age.

Keywords: L-Carnitine, performance, feed conversion ratio, broiler

S1-0004 The effect of formulation diet based on total and digestible amino acids and methionine levels on male broiler carcass traits

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Protein is one of most important part of feeding. An important part of raising chickens is feeding - feeding makes up the major cost of production and good nutrition is reflected in the bird's performance and its products. The study was conducted to evaluate performance and carcass yield of broilers fed three different methionine requirement levels: high methionine (+10% NRC), standard (NRC) and low methionine (- 10% NRC) and two ways of expressing amino acids in feed-stuffs (total or digestible amino acids). 360 one-day old broiler male (Arian Strain) in six treatments with six replications (10 male chickens) in completely randomized design in 2×3 factorial experimental method; First factor including 2 type of formulation diets based on Total and Digestible Amino Acids (TAA and DAA) of feed-stuff and second including 3 levels of methionine requirement (+10% NRC ; NRC and -10% NRC requirement of Met) were used. Birds fed +10% methionine level had the highest body weight, breast weight, thighs weight and carcass percentage ($P < 0.05$). The results of this study showed that diet formulation based on digestible amino acids significantly influenced body weight, breast yield and thighs yield and abdominal fat deposition. Treatment 4 (H Met \times DAA) promoted significantly higher, bodyweight, breast and thigh weights; carcass percentage than the other treatments ($P < 0.05$). This study showed the higher efficiency of this diet as they allow a better utilization of dietary amino acids for tissue synthesis and accretion. Thus, according to the results of this study, it can be concluded that Diet formulation based on digestible amino acids levels in feedstuffs and high methionine dietary levels improved body weight gain, carcass percentage and breast meat weight in Arian broiler male. The response to formulation based on digestible amino acids was maximized when broilers received the high methionine (+10% NRC) level diet.

Keywords: digestible amino acid, broilers, arian, carcass, methionine

S1- 0006 Effect of exogenous phytase on egg production and egg quality of spent hen

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The present study determined, assessed and quantified the exogenous phytase on egg production and egg quality of spent hens. Ninety laying hens (ISA- Brown) received iso-energetic and iso-nitrogenous diet supplemented with 0, 0.05, 0.1, 0.15 and 0.2 % phytase and reared in cages. Each layer was fed 120g feed/day from 90 to 100 weeks of age. At the inception of the study the spent hen at the age of 90 weeks had an average egg production 65.21 %. Phytase addition in diet increased ($P<0.05$) egg production (7.67, 12.17, 12.04 and 15.87 % higher than control group), egg mass output (5.5, 9.34, 7.65 and 12.72 % higher than that of control) and feed conversion, but it did not alter shell weight, shell thickness, shape index, albumen index, Haugh unit, per cent yolk and yolk color. It was concluded that reduced egg production of spent hen at older ages to some extent might have a relationship to decreased availability of phosphorus. The decreased egg production in hens at older ages could be corrected by adding appropriate phytase level in the diet. Thus, it may be possible to extend and prolonged productive life of spent hens by supplying exogenous phytase in diet. However, the effect of availability of phosphorus on egg production using larger population for a longer period may be performed to confirm the findings of the current study.

Keywords: phytase, egg production, egg quality, prolong the egg laying period

S1-0008 The effects of different levels of methionine and L-carnitine on blood metabolites and liver enzymes of female broiler chickens at 42 days

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The present study was conducted to determine the effects of different levels of methionine requirement and L-Carnitine (Iohman 20% Carnitine) on 720 one day-old female broiler chickens (Ross 308) in a completely randomized experimental design with 12 treatments and 3 replicates in 36 pens with three L-Carnitine levels (0, 75 and 150 mg/kg) and four methionine levels NRC requirement recommended (85, 100, 115 and 130 NRC%). At 42 days, 2 chickens from each replicate selected randomly for measurements of body weight, serum triglyceride (TG), cholesterol (CHOL), low density lipoprotein (LDL), high density lipoprotein (HDL), glucose (FBS), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The highest body weight ($P<0.05$) was found at the level of 115% methionine + 150 mg/kg L-Carnitine with beneficial effect on serum Glu, TG, AST and ALP. The lowest serum FBS (250.67mg/dl), Serum TG (107.33mg/dl), Serum cholesterol (90.33mg/dl) AST (198 u/l), ALT (1.58u/l), ALP (3036u/l) were found at the level of 115% methionine plus 150 mg/kg L-Carnitine ($P<0.01$). In conclusion, L-Carnitine increased body weight and decreased Serum FBS, TG and cholesterol in female broiler chickens via enhancing energy metabolism. Decreased AST, ALT and ALP could be due to protective effects of methionine and L-Carnitine on liver. L-carnitine decreased Serum fat in broiler chickens. Decreased Serum glucose may be due to metabolism regulating of L-carnitine on broiler chickens. Reduced AST and ALP could be due to decreasing effects of L-carnitine on liver free radicals and reducing liver damage. Therefore we recommend that the best combination was L-carnitine supplement on 150 mg/kg diet plus 115% methionine for preventing liver damage and control of blood glucose, reduction of free radicals in broiler chickens and optimum body weight.

Keywords: L-Carnitine, methionine, triglyceride, glucose, aspartate aminotransferase

S1-0009 The impact of methionine, lysine and threonine density on body weight and carcass characteristics of male broilers from one to ten days of age

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This research evaluates Standard Ileal Digestibility (SID) of Lysine (Lys), Methionine (Met) and Threonine (Thr) density in starter phase (1-10 d) of male broilers. At day one - 480 Ross 308 male chicks were placed in 24 floor pens, for a better understanding of the impact of increase simultaneously SID Lys, Met and Thr density on growth performance, carcass traits, visceral organs and protein efficiency ratio. Four dietary treatments (6 replicates) with different levels of SID Lys, Met and Thr density, Standard (100% NRC), Medium (115% NRC), High (130% NRC), and Very High (145% NRC) level were used in a completely randomized experimental design. Broilers fed with a High (130% NRC) diet, the body, carcass, breast and thigh weights at 10 d increased by 34.1 g (13.78%), 22.9 g (12.1%), 11.6 g (24.3%) and 5.4 g (9%), respectively, compared with the standard group ($P < 0.05$). Intestine weight and percentage decreased with supplementation diet with Lys, Met and Thr at 10 d ($P < 0.05$). Protein efficiency rate was significantly lowest in standard diet after 10 days of trial compared to other diets. The results of this study suggest that additional Lys, Met and Thr at the level of 130% of NRC (SID Lys, 14.25g/kg; Met, 6.48g/kg; Thr, 10.4g/kg) significantly optimized broiler performance, carcass, breast and thigh weight and protein efficiency rate where as intestine and proventriculus weight were significantly lowest compared with other diets at ten days of age.

Keywords: broiler, methionine, lysine, threonine, efficiency

S1-0010 The effects of different levels of L- carnitine on carcass traits and blood metabolites and liver aspartate aminotransferase (AST) of male broiler chickens at 21 day of age

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The present study was conducted to determine the effects of different levels of L- Carnitine (Iohman 20% Carnitine) on 360 one day Ross 308 male broiler chickens in completely randomized experimental design with 3 treatments and six replicates (20 male broiler chickens in each replicate). Experimental groups included 3 different L- Carnitine levels (0, 75 and 150 mg/kg). At 21 days, 2 male broiler chickens from each replicate selected randomly for measurements of body weight, carcass characteristics and blood metabolites. Body weight of male broiler chickens consuming 150 mg/kg diet of L- Carnitine (589.58 g) was greater than of the other groups ($P < 0.01$). Carcass percentage of male broiler chickens consuming 150 mg/kg diet of L- Carnitine (59.79%) was greater than of the other groups ($P < 0.05$). Breast and thigh percentage of male broiler chickens fed 150 mg/kg diet of L- Carnitine (21.18 and 17.92% respectively) was greater than of the other groups ($P < 0.05$). Abdominal fat percentage of male broiler chickens consuming 150 mg/kg diet of L- Carnitine (0.29%) was lesser than of the other groups ($P < 0.05$). Serum AST (232.58 u/l) was the least in male broiler chickens consuming 150 mg/kg diet of L- Carnitine compared with other groups ($P < 0.01$). Serum glucose concentration was lowest in male broiler chickens (240mg/dl) consuming 150mg/kg of L- Carnitine compared with other groups ($P < 0.01$). Liver weight was significantly lowest in male broiler chickens (18.25g) consuming 150mg/kg of L- Carnitine compared with other groups ($P < 0.05$). The effects of 3 different levels of L- Carnitine were non-significantly on serum concentration of triglyceride, LDL and HDL ($P < 0.05$). This study recommended that 150mg/kg L- Carnitine in male broiler diet in starter period were increasing body weight, carcass, breast and thigh percentages and decreasing abdominal fat weight, liver weight, and serum level of glucose and aspartate aminotransferase.

Keywords: broiler, carcass, blood metabolites, liver, starter

S1-0011 The effect of different combination of boiled and unboiled tomato waste (*lycopersicon esculentum*) in diet on carcass and meat quality of broiler

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The aim of this experiment to evaluate the effect of different combination of tomato (*Lycopersicon esculentum*) waste in boiled tomato (BT) waste and unboiled tomato (UT) waste in broiler diet on carcass and meat quality of broiler. Tomato waste in this experiment is rejected fresh tomato and unharvested tomato by farmer. High lycopene content in tomato could lowering cholesterol, and boiled of tomato in boiled water for 8 minutes changed the lycopene structure from trans to cis form, and lycopene in cis form is easier absorbed by poultry digestive tract in comparing with lycopene in unboiled tomato. An experiment was performed by using Completely Randomized Design with 6 different combinations of UT and BT dried tomato waste: 0%UT:0%BT, 25%UT:75%BT, 50%UT:50%BT, 75%UT:25%BT, 100% UT, and 100% BT in broiler diet, and each treatment was replicated 5 times. Measured parameters were body weight, carcass weight, carcass percentage, abdominal fat pat percentage, fat and cholesterol of thigh meat, and fat and cholesterol in liver. The result showed that body weight, carcass weight, carcass percentage, abdominal fat pat percentage, and liver fat were not affected by each combination of UT and BT ($P>0.05$), while fat and cholesterol in thigh meat, and liver cholesterol of broiler were affected ($P<0.05$). It concluded, the inclusion of 25%UT:75%BT combination of dried tomato waste in broiler diet is the effective combination in maintain the carcass performance and lowering fat and cholesterol in thigh meat, and liver cholesterol of broiler.

Keywords: boiled tomato, unboiled tomato, carcass, meat quality, broiler

S1-0012 Impact of food industry by-products and wastes of corn crunches, potato chips, and sugar beet on broilers performance

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There is a great shortage in poultry feed ingredients in Egypt; therefore, this study was carried out to evaluate the usage of some food industry byproducts and wastes in broiler diets. The effects of partial substitution of yellow corn (YC) with two levels of corn crunches waste (CCW), potato chips scraps (PCS), potato starchy waste (PSW), or sugar beet pulp (SBP) on growth performance, carcass traits and economic efficiency of broiler chicks were understudy. A total number of 297 unsexed one-day old ROSS broiler chicks were equally assigned into nine dietary groups (control and 8 treatments), each group of three even replicates. Two levels of each of the four byproducts partially replaced 5% or 10% of YC in the 8 treatments diets. Body weight (BW), body weight gain (BWG), feed consumption (FC), feed conversion ratio (FCR), and relative economic efficiency (REE) were estimated for the whole experimental period (6 weeks). At the end of the experiment, 6 birds from each group were sacrificed for evaluating different carcass traits. The results revealed that BW and BWG at 6 wks of age of the groups received 5% CCW or 10% PCS in place of YC were not significantly different from the control group; however, the lowest BW and BWG were detected for the groups received PSW or SBP. There were no significant differences in FC and FCR between all groups, except those received SBP which were worse. In regard to carcass traits, no significant differences were detected between treatments in breast % and legs % (thigh and drumstick); but eviscerated carcass weight, breast weight and legs weight were significantly lower for the groups received SBP. The best net revenue was detected for the group received CCW instead of 5% YC, while the worst net revenue and REE were detected for SBP groups. In conclusion, corn crunches waste or potato chips scraps could replace 5% or 10% YC; respectively, in broiler diets till 6 weeks of age without any adverse effect.

Keywords: corn crunches waste, potato chips scraps, potato starchy waste, sugar beet pulp, broiler performance

S1-0013 Effect of wet fermented cassava peel meal fortified with palm oil sludge on the performance and organ characteristics of broilers

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The effect of wet fermented cassava peel meal fortified with palm oil sludge on the performance and internal organ characteristics of broilers was determined in a 56-day feeding trial. The cassava peels were collected in batches, wet fermented in water for 24 hours, sun-dried for 3-5 days and ground into meal using a hammer mill. The meal was then sieved using a 2mm sieve mesh to remove more coarse root scale. The sieved meal was mixed with palm oil sludge in a ratio of 5:1. Three experimental broiler diets were formulated such that the diets contained 0, 30, and 40% cpm + pos respectively. The diets were later adjusted at the finisher phase to still contain the same levels of cpm + pos. The diets were randomly assigned to three groups of day-old Anak broilers in a completely randomized design (CRD). Each group of 30 birds was housed in three pens with ten birds per pen. Data on performance and internal organ characteristics were collected and calculated. Data generated were subjected to the one way analysis of variance ANOVA. Differences between means were determined using standard error mean at 5% probability. The birds were fed ad libitum for 56-days. There was no significant ($P > 0.05$) difference in the feed intake and feed conversion ratio among Broiler birds fed cassava peel meal fortified with palm oil sludge compared to the control group at both the starter and finisher phase. Weight gain was not significantly ($P > 0.05$) affected by all the experimental diets in both starter and finisher phase while feed cost and cost/kg meat decrease with increasing dietary inclusion of the meal. There was no significant ($P > 0.05$) difference in the internal organ weight of birds fed cpm + pos at both 30% and 40% levels compared to the control group except for the heart and kidney weight. It is concluded that 24 hour wet fermented cassava peel meal fortified with palm oil sludge could be incorporated into broilers diets at 40% level replacement of maize.

Keywords: cassava, broilers, performance, organ

S1-0014 The effect of L- carnitine on morbidity and yolk sac absorption in broiler chickens up to ten days

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This study was conducted to evaluate the four dietary L- Carnitine levels on morbidity and yolk sac of broiler chickens Ross 308. 240 day old Ross 308 broiler chickens were reared under optimum growth condition until 10 days of age. They were randomly distributed into 12 floor pens in a completely randomized experimental design (4pen/treat, 20birds/pen). L-Carnitine levels used in this experiment were control, 100, 200 and 300 ppm. Sample of 96 chicks were used to weight residual yolk sac at 1, 3, 7 and 10 d of age and numbers of chicks showing morbidity signs during the first week of life were recorded. Morbidity was defined as chick showing sign of diarrhea in cloak area. All the chicks were checked individually within each replication. For each group, numbers of chicks that showed sign of diarrhea were recorded. These numbers were used to calculate the proportions of chicks showing morbidity signs as Morbidity. L-Carnitine had significant effect ($P < 0.05$) either on absorption yolk sac, but had little significant differences between treatments that had received L- Carnitine. L-Carnitine had significant effect ($P < 0.05$) on reduction morbidity post hatch. Results concluded that L- Carnitine supplementation in diet leads to quick utilization of yolk sac content improved in a dose dependent way, and may hence be considered for improving early post-hatch performance.

Keywords: yolk sac, morbidity, broiler, L-Carnitine, post hatch

S1- 0015 Nutritional evaluation of differently processed piper (canavalia plagioperma) seed meal (cpsm) in broiler starter diets

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Nutritional evaluation of differently processed piper (Canavalia plagioperma) Seed meal (CPSM) in broiler starter diets Esonu, B.O*, Izukanne, R.O, Udedibie, A.B.I. and Okeudo, N. J. Department of Animal Science and Technology, Federal University of Technology Owerri *Corresponding author: E-mail esonubabs@yahoo.com. Abstract A feeding trial was conducted with the aim of improving the nutritive value of Canavalia plagioperma seedmeal (CPSM) for broiler starter rations. One hundred and fifty kilogram (150kg) of Canavalia plagioperma seeds were cracked and thereafter divided into three batches. Two batches were soaked in water while the third batch was soaked in alkaline solution (2 % palm bunch ash by weight of the cracked seeds) in different plastic containers for 48 hours, before draining off the soaking solutions. The first, second and third batches were boiled in different pots for 1 hour, 2 hours and 1 hour respectively. They were separately sundried for 4 - 5 days and milled to produce cracked soaked in water and boiled for 1 hour (CSWB1), cracked soaked in water and boiled for 2 hours (CSWB2) and cracked soaked in alkaline and boiled for 1 hour (CSAB1), Canavalia plagioperma seedmeals (CPSM) respectively. The meals were used to formulate seven broiler starter rations at 0 %, 10 % and 20 % dietary inclusion, levels, respectively (Table 3). Each of the seven broiler starter rations was fed to a group of 42 one-week-old broiler chicks for 28 days, to evaluate the performance of the birds and economic implications. There were significant differences ($P<0.05$) in daily feed intake, daily body weight gain and feed conversion ratio. Results from this trial suggests that CPSM could be included in broiler starter diets up to 20 % without adverse effect if cracked, soaked in water and boiled for an hour. Key words: Canavalia plagioperma seeds, broiler starter, cracked, soaked, alkaline, boiled.

Keywords: canavalia plagioperma seeds, broiler starter, cracked, soaked, alkaline, boiled.

S1- 0016 Anaerobic fermentation of rice bran using rumen inoculate for desirable chemical changes as feed for livestock

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Rice bran (RB) and deoiled rice bran (DORB) is a by product of rice-milling industry which is cheap and using as animal feed throughout the world. Considering limitation like higher content of fiber and lower availability of few micronutrients including phosphorus they were fermented anaerobically using inoculum from rumen of a sheep to get fermented value added feed ingredient. Initially they were fermented for 12 hours at 390C giving 10, 20, 30, 40, 50 and 60% moisture and addition of 10% rumen inoculum. Then DORB was fermented for 24, 48 and 72 hours at 390C and giving 10% inoculate but different moisture level (10, 20, 30, 40, 50 and 60%). Before and after fermentation they were analysed for proximate components, NDF, total-P, inorganic-P and phytate-P. Fermentation of RB and DORB for a period of 12 hours decreased pH, CF, NDF and phytate-P, but increased inorganic- P content ($P<0.05$). Further fermentation of DORB also showed similar result for lowering pH, CF, NDF and phytate-P, but increased inorganic- P ($P<0.05$). So, inoculation of rumen microbes reduce phytate-P and fiber content (CF and NDF) of RB as well as DORB. Further research is needed for confirmation and bioavailability in nonruminant animals.

Keywords: anaerobic, fermentation, rice bran, feed, livestock

S1- 0017 Effect of different dietary inclusion levels of Biotronic® SE on the performance of broiler chickens

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A feeding trial was conducted to evaluate the effect of inclusion levels of Biotronic® SE (BSE) in the diets of broiler chickens and effect on performance. Two hundred and forty broiler chickens were randomly assigned to four experimental diets with three replicates per treatment. At the starter phase (0-4 weeks) T1, T2, T3 and T4 were supplemented with 0g, 300g, 400g, and 500g BSE per 100Kg diet respectively, while at the finisher phase (4-8 weeks) T1, T2, T3 and T4 was supplemented with 0g, 200g, 300g, and 400g BSE per 100 Kg diet respectively. Growth parameters taken include initial weight, final weight, feed consumption, FCR, feed cost/Kg and feed cost/Kg gain. Carcass quality was evaluated at eight weeks and the pH of intestinal organs was also measured using 3 birds per treatment. All data generated were subjected to analysis of variance and difference in means was compared using Duncan multiple range test. From the result of the study, birds fed diets containing 300g/100kg BSE showed best performance in terms of weight gain, FCR and feed cost/Kg gain at the starter phase. However at the finisher phase birds supplemented with 400g/100Kg BSE showed best performance in terms of FCR and feed cost/Kg gain. This is 100g/100Kg feed above maximum recommended level of 300Kg/100 Kg feed Significant ($P < 0.05$) differences existed for breast and back cut parts but with no specific trend and similarly for intestinal length and gizzard weight, but all other carcass parameters were not significantly ($P > 0.05$) different. The pH values for the intestinal organs showed no significant ($P > 0.05$) difference for crop, duodenum, jejunum and ileum but significant ($P < 0.05$) for proventriculus, gizzard, caecum, large intestine and liver. It may be concluded that supplementation of broiler diets with 300g/100kg of feed at starter phase and 400g/100kg at finisher phase improves broiler performance and significantly reduced cost of production.

Keywords: Biotronic® SE, performance, carcass quality, production cost

S1- 0018 Efficacy of an anti- myco-toxin additive in preventing the toxicity of aflatoxin in ducks

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The objective of this experiment was to evaluate the efficacy of an anti-mycotoxin additive (Mycoad) in preventing the negative effects of natural aflatoxin (AF) in ducks. Two hundred and forty one-day-old meat-type ducks (Cherry Valley) were placed on floor pens with continuous light and randomly distributed into 6 treatments with 2 replications of 20 ducks each. Ducks were fed a corn-soy diet tested free of mycotoxins; using a 300 ppb natural AF corn to generate the contaminated diets. Treatments were: < 30 ppb AF (control); < 30 ppb + 0.25% Mycoad; 120 ppb AF; and 120 ppb AF diet supplemented with 0.15, 0.25 and 0.35% Mycoad. Feed and water were provided ad libitum. At 4 weeks of age, productive performance, organs conditions and blood parameters were measured. All data were submitted to ANOVA and differences between means compared by Duncan's test ($P < 0.05$). Results showed that ducks fed 120 ppb AF presented significant lower body weight gain (BWG) (-13.7%), reduced feed intake (-6.5%), poorer feed conversion (FC) (+8.8%) and increased mortality (+10.3%) compared to ducks fed control diet. Significant deleterious effects of AF were observed also in feather growth, eye necrosis, web-toe hemorrhage, leg deformity, tibia bone porosity, liver paleness and fat content, organ weights, hematology and serum biochemical values. The addition of 0.15% Mycoad decreased mortality and partially recovered the toxic effects of 120 ppb of AF (+9% BWG). Supplementation of 0.25% Mycoad was required to significantly ($P < 0.05$) prevent all the deleterious effects of AF (+16% BWG; -6% FCR). No further protection was observed with 0.35% Mycoad. Results indicated that 0.25% Mycoad completely prevent the toxic effects of 120 ppb natural AF on performance, chronic toxicity, target organ, and blood parameters in ducks. Also, inclusion of 0.25% Mycoad to the control diet did not affect any parameter measured, demonstrating that the product does not interfere with nutrient absorption.

Keywords: Mycoad, aflatoxin, ducks, performance, organs

S1-0019 Effect of substitution of fermented sunflower seed (*helianthus annuus* L.) to soybean meal on production and egg quality from native laying hens

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This study aims to determine the level of use of fermented sunflower seed (*Helianthus annuus* L.), to substitute 100% soybean meal in native laying hen rations without adverse effect on laying hen production. The study consisted of a series of field experiments to study the biological effects, production and on quality of eggs. The variables measured were feed intake, feed conversion, and income over feed cost (gross profit) as well as variables related to egg production (Hen day production and egg weight) and thickness of eggshell and yolk color index for quality of eggs. Two hundred and forty laying hen aged 20 weeks were used in this experiment. The completely randomized design was assigned with six treatments to replace 0, 20, 40, 60, 80 and 100% of soybean meal with fermented sunflower seed with four replications. Data were analyzed statistically using ANOVA, and if the result shows a marked influence, it will followed by Duncan's Test (DMRT). The results showed that the performance of laying hen had not affected significantly by substituting soybean meal fermented sunflower seed. Feed intake, feed conversion and egg production is not influenced either. In summary, the use of fermented sunflower seed to substitute soybean meal in the ration can be up to 100% in the diet of laying chicken.

Keywords: fermented sunflower (*Helianthus annuus* L.) substitution hen day production native laying hen

S1-0021 Bio-transference of aflatoxin B1 from feed to the breast of hens as aflatoxin M1

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The presence of hydroxylated metabolites of aflatoxin B1 (AFB1), such as AFM1 has been reported in the breast of layer hens, once they have reached their laying cycle and been sent to slaughter. In Mexico the consumption of hens has great demand, mainly in the south east of the country. Considering that AFM1 is limited in milk at levels of 0.5 to 0.05 µg/L, depending on the regulations of each world region, it was proposed to make an evaluation of the bio-transference of AFB1 present in a diet contaminated with 500 µg/kg (ppb) and to quantify the presence of AFM1 in the breast, after a week of consumption. At the same time an anti-mycotoxin agent was included to verify its efficiency in this experiment. Material and methods: 45 ninety week old hens were selected, and were divided in three experimental groups (15 hens per group) and were placed in individual cages. Three diets were elaborated: negative control group with no AFB1 and no adsorbent; positive control group with 500 ppb of AFB1 and challenge group with 500 ppb of AFB1 and the equivalent of 5kg/t of a commercial anti-mycotoxin agent. Birds were sacrificed after 8 days of treatment. Breasts were removed and the content of AFM1 was quantified individually through a system of Ultra High Performance Liquid Chromatography (UHPLC). Results: The concentration of AFM1 in the breasts of the negative control group was lower than the limit of detection of 0.001 ppb. In the breasts of the positive control group the concentration was 0.004 ppb, and in the challenge group the concentration was 0.002. Discussion and conclusion: The obtained results show that it is minimal bio-transference of AFB1 to the breast as AFM1, therefore this feed is considered as innocuous, with respect to the contamination with mycotoxins. The efficiency of the adsorbent was 50 %.

Keywords: innocuity, breast, aflatoxin B1, aflatoxin M1, antimycotoxins agents

S1-0022 Influence of dietary graded levels of digestible lysine and lowering energy to protein ratio on the growth performance of broilers under subtropical summer conditions

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The digestible lysine requirement for the growth performance of broilers fed diets with decreasing energy to protein ratio and reared under subtropical summer conditions were determined. Treatments consisted of five wheat-based diets with five levels (0.90, 0.95, 1.00, 1.05 and 1.10%) of digestible lysine (DL) and lowering energy to protein ratio (EPR; 13.6, 12.8, 12.0, 11.4 and 11.0), with five replicates of 80 birds each (n = 2000). Body weight gain (BWG) significantly increased by increasing DL levels during wk 1 ($P < 0.001$), while 1.05% DL promoted the highest BWG during the whole experimental period ($P < 0.01$). Increasing DL level resulted in significant reduction in feed intake (FI) and BWG to DL intake ratio (BWG:DLI) during wk 1-3 ($P < 0.05$). Feed:gain ratio (FGR) significantly ($P < 0.01$) improved with DL levels up to 1.05% during the whole experimental period, except for wk 5. The increasing ambient temperature had a linear effect on the all growth parameters. It is inferred that best growth performance can be obtained at 1.05% DL, maintaining all other AA constant under subtropical summer conditions.

Keywords: digestible lysine, subtropical summer, wheat-based diets, broiler

S1- 0023 Effect of xylanase enzyme on metabolizable energy partitioning in broiler chickens fed wheat-based diets

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The objective of this study was to determine the effect of xylanase supplementation on efficiency of energy utilization in broiler chickens fed wheat-based diets. A total of 120 Ross 308 male broilers were divided into 2 treatment groups (without or with 500 mg/kg xylanase supplementation) in 5 replicates of 2 birds each, from day 22 up to day 42 of age. The five feed intake treatments consisted of ad libitum, 85, 70, 55, and 40% of ad libitum intake. The actual ME intake was quantified by measuring the feed residuals during the experiment. A 10- d feeding trial (from 25 to 35 d of age) was conducted to determine the AME of the experimental diet by using a total collection technique. The influence of xylanase enzyme on maintenance ME requirements (ME_m) was determined by a 21 d experiment, using the comparative slaughter technique. Average daily gain (ADG), average daily feed intake (ADFI), carcass composition, ME intake (MEI), total energy retained (TER), energy retained as fat (ERF) and protein (ERP), and efficiencies of energy utilization were measured. Xylanase did not affect efficiency for fat retention (kf) but significantly increased ADG, carcass fat and protein contents, body energy, TER, ERF, ERP, efficiency of energy utilization for gain (kg) and maintenance (km), and efficiency for protein retention (kp). In addition, xylanase supplementation decreased ADFI, MEI, and ME_m requirements. The method of calculating ME_m seems to greatly influence the partition of energy between maintenance and growth as fat and protein and by inference efficiency of energy use. The results from current study can be used in calculation of the energy requirements for growing broilers fed wheat-based diets and especially for finishing birds from 22 to 42 d of age.

Keywords: broilers, energy partitioning, wheat, xylanase

S1-0024 Effect of feed form and citric acid on partitioning of retained energy in broiler chickens fed wheat-based diets

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The aim of this study was to determine the effect of citric acid (CA) and feed form in broiler chickens fed wheat-based diets on energetic efficiency, energy partitioning, carcass composition and growth performance from 22 to 42 d of age. Two hundred 22-d-old Ross 308 male broilers were allocated in 4 experimental treatments, each of which was replicated 5 times. A 2×2 factorial design was used in the study, and the main factors were CA (0 and 30 g/kg) and feed form (mash and pellet). The five feed intake treatments consisted of ad libitum, 85, 70, 55, and 40% of ad libitum intake. The actual ME intake was quantified by measuring the feed residuals during the experiment. A 10-d feeding trial (from 25 to 35 d of age) was conducted to determine the AME of the experimental diet by using a total collection technique. The influences of CA and feed form on maintenance ME requirements (ME_m) were determined by a 21 d experiment, using the comparative slaughter technique. Average daily gain (ADG), average daily feed intake (ADFI), carcass composition, ME intake (MEI), total energy retained (TER), energy retained as fat (ERF) and protein (ERP), and efficiencies of energy utilization were measured. The acidified and pelleted diets significantly increased ADG, carcass fat and protein contents, body energy, TER, ERF, ERP, efficiency of energy utilization for gain (kg). In addition, pelleted diet decreased ADFI, MEI, and ME_m requirements and increased efficiency of energy utilization for maintenance (km), and efficiencies for protein retention (kp) and fat retention (kf). Citric acid decreased efficiency for fat retention (kf) but did not have a significant effect on ME_m requirements. The results from this experiment provide important data about the efficiency of ME utilization for protein deposition which will be used to improve the net energy system in broilers.

Keywords: broilers, citric acid, feed form, energy partitioning, wheat

S1-0025 Partitioning of retained energy in broilers subjected to cyclic heat stress

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An experiment was conducted to study the utilization of ME by broilers reared under cyclic heat stress from 22 to 42 d of age. Two hundred 22-d-old Ross 308 male broilers were allocated in 4 experimental treatments, each of which was replicated 5 times. They were fed amounts of feed that ranged between ad libitum and 40 to 85% ad libitum each day. A 2×2 factorial design was used in the study, and the main factors were sex (male or female) and temperature (thermoneutral zone or heat stress). From d 22 to 42, birds were either raised in a thermoneutral zone (22°C) or subjected to cyclic heat stress by exposing them to 33°C for 10 h (from 0700 to 1700) and 22°C from 1700 to 0700. The actual ME intake was quantified by measuring the feed residuals during the experiment. A 10-d feeding trial (from 25 to 35 d of age) was conducted to determine the AME of the experimental diet by using a total collection technique. The influences of heat stress and sex on maintenance ME requirements (ME_m) were determined, using the comparative slaughter technique. Data were collected to determine ME intake (MEI), total energy retained (TER), energy retained as fat (ERF) and protein (ERP), heat production (HP), and efficiencies of energy utilization. TER, ERF, and ERP per unit metabolic BW, MEI, efficiency of energy utilization for gain (kg) and maintenance (km), and efficiency for protein retention (kp) were higher for males. During heat stress, ME_m for females was lower than for males. Heat stress decreased MEI, efficiency of energy utilization for gain (kg) and maintenance (km), and efficiency for protein retention (kp), but increased ME_m requirements, TER, ERF, ERP, and efficiency for fat retention (kf). Because ME intake for maintenance requirements represents a large portion of the ME intake, the results from this experiment could be considered in calculation of energy requirements for heat stressed-growing broilers.

Keywords: broiler, heat stress, energy requirement, metabolizable energy

S1- 0026 Bioefficacy of methionine hydroxy analogue- FA compared to DL- methionine on egg production performance, nutrient utilization and immunity in laying Japanese quails

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Bio- efficacy of methionine hydroxy analogue-FA (MHA) to dl-methionine (DL-Met) was compared in laying Japanese quails (n=250), divided into 125 groups of two each. A basal diet (T1) was formulated (CP 190 g, ME 12.13 MJ, 3.4 g Met and 3.8 g Cys /kg diet). In second (T2) treatment 1.1 g/kg synthetic DL-Met was added to achieve a dietary level of 4.5 g/kg, whereas 1.64 (T3), 1.86 (T4) and 2.53 (T5) g/kg MHA was added in a basal diet (T1) assuming 100, 88, and 65% efficacy to that of Met. Each diet was offered to 25 groups and fed from 56-140 d of age. Egg weight (P<0.005), hen-day production (P<0.009), FCR (per unit egg mass, P<0.04), Cell-mediated immune response (CMI, P<0.004) and Internal Quality Unit (IQU, P<0.001) improved on Met or MHA supplementation. However, Net FCR (feed: egg mass plus body weight gain) and egg quality parameters were not affected due to diets. In conclusion, lower doses of MHA (1.64 g/kg) can be included in diet to acquire maximum production potential from laying Japanese quail; however it is difficult to make any decision regarding bioefficacy of MHA compared to Met from the present study.

Keywords: methionine, MHA, egg weight, egg production, laying quails

S1- 0027 Effect of probiotic (*Pedio-coccusacidilactici*) on blood lipid constituents, and gut morphology of broilers fed vegetable oil-supplemented diets

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The present trial was carried out to elucidate the effects of probiotic and antibiotic (virginamycin) supplementation in diets containing canola (CAN), or pumpkin seed oils (PKS) on performance, blood lipid constituents and morphological parameters of the duodenum and ileum. Four-hundred and eighty, day old male broiler chicks were randomly placed in a 2 × 3 factorial arrangement of dietary treatments including the two fat sources (CAN and PKS) and the two additives (without additive, probiotic, and virginamycin) with 5 replicates per treatment. No effect of fat sources or additives on the performance traits and abdominal fat were found. PKS significantly improved triglyceride, high density lipoprotein (HDL) and very low density lipoprotein (VLDL) levels (P<0.01), duodenal villus height and surface area (P<0.05). Likewise the probiotic improved (P<0.01) triglyceride, total cholesterol, and VLDL levels, and increased (P<0.01) both ileal villi height and the ratio of villus height to crypt depth when compared with the non-dietary additive group at d 42. On the other hand, virginamycin significantly decreased (P<0.01) total cholesterol and low density lipoprotein (LDL) levels. However, the probiotic interacted positively with PKS leading to increases (P<0.01) in ileal villi height and the ratio of villus height to crypt depth, triglyceride, total cholesterol and VLDL levels at d 42. In conclusion, PKS and the probiotic both exhibited potential improvements for bird health, as indicated by modification of some blood lipid levels and enhancement of some of the morphological parameters in the small intestine. Therefore, the addition of probiotics to fat-supplemented diets can be recommended when the linoleic-oleic group of vegetable oils are used.

Keywords: blood fat, broiler, intestinal structure, *Pediooccusacidilactici*, vegetable oil

S1- 0028 Growth performance of broiler chicken fed varied density nutrient diet supplemented with direct fed microbial

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Comparative dietary response of the different strains of Lactobacilli, Streptococci and Yeast isolated from leopard feces (*Panthera leo*) was studied as direct fed microbial (DFM) in poultry broiler birds reared on low density crude protein formulated test diet D2. Various treatments consisted of T0 (Control, culture medium) offered standard formulated diet D1, T1 (Control, culture medium) and T2 (Lactobacillus casei + Streptococcus fecalis + Saccharomyces cerevisiae) offered lower crude protein density test diet D2. Supplementation of isolated DFM improved the growth performance only in terms of better feed conversion ratio and numeric increase in live weight gain of the broiler birds of treatment T2 compared to its control T1 offered test diet D2 both during starter and finisher phase but could not match the growth performance of control T0 offered standard formulated diet D1. Treatment group T2 exhibited better intestinal micro flora balance, effective colonization and higher microbial count in the intestinal tract and lower ($P<0.05$) blood cholesterol and glucose with higher ($P<0.05$) nitrogen retention in the digestibility studies. It was thus concluded that supplementation of isolated DFM supported the growth performance of poultry broiler birds offered low density crude protein test diet D2.

Keywords: probiotics, DFM, chicken, mineral, feed, microbes

S1-0029 Effects of replacing soybean meal with rapeseed meal and corn with sweet potato powder on growth rate, blood metabolites and intestinal morphology of Japanese quails

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The objective of this study was to evaluate the effect of replacing soybean meal with rapeseed meal and corn with sweet potato powder on performance, blood parameters and carcass traits of quails. A total of two hundred day old male Japanese quails were randomly assigned to five dietary treatments and four replicates (each with 10 birds) from 10 to 45 days. All diets were adjusted to nutrient requirements of Japanese quails. Diets were containing 0, 10 and 20% rapeseed meal and/or sweet potato powder. The growth performance including feed intake, body weight gain and feed conversion ratio were not affected by dietary treatments ($P>0.05$). At 45 days of age, the carcass traits including carcass yields and organ weights did not showed significant effect. At 45 days of age, the diets fed containing different levels of rapeseed meal and/or sweet potato powder were not effected on blood metabolites (except of triglyceride). The serum triglyceride was lower in diet fed contained 20% rapeseed meal- 20% sweet potato powder than the diet fed contained 10% rapeseed meal- 10% sweet potato powder. The intestinal morphology including villi length and villus absorptive surface area were not affected by dietary treatments. The results indicated that the replacing of soybean meal with rapeseed meal and corn with sweet potato powder in quails diet did not adverse effects on growth performance and carcass traits.

Keywords: growth, intestine, potato, quails, rapeseed

S1- 0032 Effects of different mycotoxin adsorbent products on performance, egg trace elements content and serum biochemical parameters of laying hens

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This experiment was conducted to study the effects of adsorbents on performance, egg trace elements content and serum biochemical parameters of laying hens. A total of 135 healthy 38-week-old laying hens were obtained and randomly divided into 3 groups with 5 replicates per group and 9 hens per replicate. Laying hens in the control group were fed a basal diet, and laying hens in experimental groups were fed the diets supplemented with 5 g/kg two kinds of adsorbents, respectively. The adjustment period lasted for 7 days, and the experimental period lasted for 42 days. The results showed as follows: 1) Compared with the control group, the laying rate of B group in period of 3~5 weeks was significantly increased ($P < 0.05$). 2) Compared with the control group, the yolk index of C group in the 35th days was significantly increased ($P < 0.01$). 3) Compared with the control group, the content of copper and manganese in yolk of experimental groups in the 14th and 35th days was no significant difference ($P > 0.05$). The content of zinc in yolk of B group in the 14th days was significantly increased ($P < 0.05$). 4) Dietary supplemented with adsorbents had no significant effect on the serum GSH-Px activity and the serum MDA content in the 14th and 35th days ($P > 0.05$). Compared with the control group, the serum T-AOC of experimental groups in the 14th was significantly increased ($P < 0.01$); the T-SOD activity of C group in the 35th days was significantly increased ($P < 0.05$). 5) Compared with the control group, the ALP activity, Glu content in serum of B group was significantly decreased ($P < 0.05$), the trend of Fe, Ca content in serum were increased, and the trend of UA content in serum was decreased ($P > 0.05$); the TC content in serum of C group was significantly decreased ($P < 0.05$). Thus, Dietary supplemented with adsorbents had increased performance of laying hens and the antioxidant capability of serum, also improved serum biochemical parameters.

Keywords: laying hens, mycotoxin adsorbents, performance, trace elements, antioxidant

S1- 0033 Growth performance and ammonia concentrations in broilers fed on two levels of dietary chitosan

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The objective of this experiment aimed to investigate the effect of two levels of dietary chitosan on growth performance and ammonia concentrations in broiler production. One-day old male Ross 308 broilers ($n=392$) were allocated into four groups of seven replicates (14 chicks each) in a completely randomized design (CRD). All broilers were raised in the open house system (1.5 m² per pen). Broilers in control group (group 1) were fed on commercial basal diet composed of corn-soybean meal as the major ingredient while those in group 2 received 200 ppm amoxicillin supplemented in basal diet. Chitosan was added in basal diet at the levels of 1 and 2 g/kg in groups 3 and 4, respectively. Feed and water were provided ad libitum. Ammonia concentrations were measured above the litter surface using an ammonia detector. The data (i.e. feed intake, weight gain, mortality rate and ammonia concentrations) were recorded at 21 and 39 d of age. Data were analyzed using one way ANOVA and Duncan's multiple range tests. The overall average daily gain (ADG) and feed conversion ratio (FCR) in group 2 were significantly better ($P < 0.05$) compared with those of group 3 but there were no significant difference between control group and those supplanted with chitosan (groups 3 and 4) at 21 and 39 d of age. Live weight of birds in group 2 at 21 d of age were significantly greater ($P < 0.05$) compared with that of group 3 while no significant difference was found between control group and both chitosan groups. There was no significant difference of live weight among groups at 39 d of age. Ammonia concentrations in group 4 were significantly lower ($p < 0.05$) compared with that of group 1 at both ages. In conclusion, the addition of chitosan at 2 g/kg in broiler diet tended to improve FCR at 39 d of age as similar to antibiotic growth promoter. Moreover, it reduced ammonia concentrations at 21 and 39 d of age.

Keywords: chitosan, broilers, performance, amoxicillin, ammonia

S1- 0035 Odour flux from litter of broiler chickens fed diets differing in protein levels and additives

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The effect of dietary crude protein (CP) and additives on odour flux from broiler litter was investigated using 180 day-old Ross 308 male chicks randomly allocated to five dietary treatments with three replications of 12 birds each. A 5×3 factorial arrangement of treatments was employed. Factors were: diet (low CP, high CP, high CP+antibiotic, high CP+probiotic, high CP+saponin) and age (15, 29, 35 days). Low CP (LCP) and high CP (HCP) diets differed in CP levels by 4.5-5%. The low CP diets were supplemented with L- valine, L- isoleucine, L- arginine, L- lysine, D,L- methionine and L- threonine. The antibiotic used was Zn Bacitracin, the probiotic was a blend of three *Bacillus subtilis* strains and the saponin came from a blend of *Yucca* and *Quillaja*. Odorants were measured from litter headspace using a flux hood and selective ion flow tube mass spectrometry (SIFT-MS). Results were log transformed and analysed by two-way ANOVA with repeated measures using JMP statistical software v.8, and means were separated by Tukey's HSD test at $P < 0.05$. The results showed that LCP group produced lower flux of dimethyl amine, trimethyl amine, H_2S , NH_3 and phenol in litter compared to HCP group ($P < 0.05$). Similarly, HCP + probiotic group produced lower flux of H_2S ($P < 0.05$) and HCP + saponin group produced lower flux of trimethylamine and phenol in litter compared to HCP group ($P < 0.05$). The dietary treatments tended ($P = 0.065$) to have higher flux of methanethiol in HCP group compared to others. There was a diet x age interaction for litter flux of diacetyl, acetoin, 3- methyl- 1- butanol, 3- methylbutanal, ethanethiol, propionic acid and hexane ($P < 0.05$). Concentrations of diacetyl, acetoin, propionic acid and hexane in litter were higher from LCP group compared to all other treatments on d 35 ($P < 0.05$) but not on days 15 and 29. Thus, the low CP diet, *Bacillus subtilis* based probiotic and *Yucca/Quillaja* based saponin were effective in reducing the emissions of some key odorants from broiler litter.

Keywords: broiler, crude protein, additives, odour, SIFT-MS

S1-0037 Variations in starch and energy utilization and growth performance of broiler chicks fed diets containing starch from different sources

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The effect of starch from different cereal and tuber sources on energy utilization, starch digestibility and growth performance of broiler chicks was investigated. Starch from maize, millet, wheat, sweet potato and cassava were extracted by wet milling and incorporated at $46 \pm 2\%$ of broiler starter diets as the primary energy source. Ninety 8d-old chicks were allotted to 15 battery brooder units; 5 treatments, 3 replicates, 6 birds per replicate in a completely randomized design. Weight gain (WG), feed intake (FI) and gain: feed (G:F) were monitored during the study. Metabolic studies were carried out on 24-27d in metabolic cages. On 28d, birds were slaughtered and digesta collected from the duodenum (D), posterior jejunum (PJ) and posterior ileum (PI). Digesta and feed were dried at 50°C and analysed in triplicates for starch and gross energy. Data were analysed using ANOVA and means separated using DMRT. Starch digestibility varied ($P < 0.05$) among the different diets in the D, PJ and PI with higher starch digestibility recorded for wheat (83.35, 93.95 and 95.77%) compared to millet (73.81, 82.84 and 85.98%). However the diets did not affect ($P > 0.05$) total tract starch digestibility. Energy digestibility varied ($P < 0.05$) in the D and PJ with highest values obtained for cassava starch (80.21 and 83.35%) and least for millet starch (60.67%) in the D and maize starch (72.95%) in the PJ, but not in the PI. Total tract energy digestibility also varied ($P < 0.05$) amongst test diets. WG at 29d varied ($P < 0.05$) among diets with higher WG in the wheat (512.28g) and least in the sweet potato diet (401.92g) while FI and G:F were not affected by the test diets. In vivo kinetics of starch, energy digestibility and weight gain varied among the different energy sources tested.

Keywords: starch digestion, energy utilization, growth performance, broiler chicken

S1-0038 Effects of small peptide chelate iron on performance,egg quality, egg yolk Fe content and serum antioxidant indices of laying hens

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This experiment was conducted to study the effects of small peptide chelate iron on performance,egg quality,egg yolk Fe content and serum antioxidant indices of laying hens.A total of 180 healthy Lohmann pink-shell laying hens(38 weeks of age) were randomly divided into 4 groups with 5 replicates per groups and 9 hens per replicates.Group A is normal control group fed basal diet ,Hens in the experimental groups for B,C,D were fed the basal diet supplemented with 60, 120, 180 mg/kg Fe as small peptide chelate iron.The adjustment period lasted for 7 days and the experimental period lasted for 42 days. The results showed as follows: 1)Dietary supplemented with small peptide chelate iron had no significant effects on the average daily feed intake,average egg weight, laying rate and feed/egg ratio ($P>0.05$).2)The experimental groups had no significant effects on egg shell strength, egg shell thickness,egg shape index,yolk index, yolk colour and haugh unit.3)The experimental groups significantly increased egg yolk Fe content($P<0.01$),the 21st day increased by 68.32%, 73.73% and 83.26% respectively, the 42nd days increased by 78.58%,82.35%and 82.96% respectively.4)Group C and group D significantly increased total superoxide dismutase(T-SOD)activity($P<0.01$)in the 42nd days.Dietary supplemented with small peptide chelate iron had an decreasing trend on the serum malonaldehyde (MDA)content($P>0.05$).In conclusion,the optimum adding level of Fe as small peptide chelate iron is 60 to 120 mg/kg.

Keywords: laying hens, small peptide chelate iron, production performance, egg quality, egg yolk Fe content, antioxidant

S1- 0039 Effects of nano- selenium on performance,serum biochemical indice and egg selenium content of laying hens

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This experiment was conducted to study the effects of nano- selenium on performance,serum biochemical indices and egg selenium content of laying hens.A total of 270 hens(200 to 210 days of age) were randomly allotted to 6 groups with 5 replicates in each group,and each replicate contained 9 hens.Hens in the control group were fed basal diet,and hens in experimental groups were fed the diets supplemented with 0.1、0.2、0.3、0.4、0.5mg/kg nano-selenium,respectively.The adjustment period lasted for 7 days,and the experimental period lasted for 63 days.The results showed as follows:1)Compared with the control group , dietary supplemented with nano- slenium increased egg number and declined feed to egg ratio ($P>0.05$).2) Compared with the control group , the content of IgM and IgA in serum of 0.4 mg/kg nano-selenium group in the 42nd days was significantly increased($P<0.05$).3) Compared with the control group , the content of ALB,TP were significantly increased and the content of TG,UA and Glu were significantly decreased of 0.3,0.4 and 0.5 mg/kg nano-selenium groups.4) Compared with the control group,the yolk selenium content of 0.5 mg/kg nano-selenium groups was significantly increased($P<0.01$).

Keywords: laying hens, nano-selenium, performance, serum biochemical, yolk selenium content

S1- 0043 Effects of different energy to protein ratios in diets of broiler breeders on progeny performance and processing yields

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Besides many factors also nutrition of the parents can affect the performance and processing yields of progeny. Therefore, an experiment was carried out to determine the effects of different energy to protein ratios in diets of broiler breeders (BB) on progeny performance and processing yields. A $2 \times 3 \times 2$ factorial design was used with 2 dietary protein levels (high = CPh; low = CPI) during rearing; 3 dietary energy levels (3,000 = MEh1; 2,800 = MEs1; 2,600 = MEI1) during the first phase of lay; and 2 dietary energy levels (2,800 = MEs2; 3,000 = MEh2) during the second phase of lay. A total of 2,880 one-day old Ross 308 BB female chicks were placed in 36 pens. At 28 and 53 wk of age 150 eggs per pen were collected and set in an incubator. After feather sexing, 10 male and 10 female chicks from the same breeder pen were randomly allotted to 36 floor pens. Body weight of the broilers in each pen was determined at d 0, 10, 17, 27, and 34. Feed intake, BW gain, and feed conversion ratio (FCR) in each pen were recorded and calculated. Mortality and health were recorded daily. At d 34, 2 random broilers per pen were identified by wing tags, and after slaughtering processing yields of these birds were measured. The data were analysed by the REML variance component analysis procedure. Progeny of 28 wk old BB fed the CPh diet showed a tendency to an improved FCR compared to BB fed the CPI diet. Feed intake tended to be lower for progeny of 28 wk old breeders fed the MEI1 diet compared to BB fed the MEh1 and MEs1 diets. A lower mortality (0.6 vs. 1.9%) and higher EPI (European Production Index) (462 vs. 450) was observed for progeny of 53 wk old BB fed the MEh2 diet compared to BB fed the MEs2 diet. No effects on processing yields of progeny of 28 and 53 wk old breeders were found. This experiment showed positive effects on progeny performance when breeders were fed a high protein diet during rear or a high energy diet during the second phase of lay.

Keywords: broiler breeders, feeding strategies, progeny, performance, processing yields

S1- 0044 Effect of probiotic (*Pediococcus acidilactici*) on blood lipid constituents, and gut morphology of broilers fed vegetable oil- supplemented diets

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The present trial was carried out to elucidate the effects of probiotic and antibiotic (virginamycin) supplementation in diets containing canola (CAN), or pumpkin seed oils (PKS) on performance, blood lipid constituents and morphological parameters of the duodenum and ileum. Four-hundred and eighty, day old male broiler chicks were randomly placed in a 2×3 factorial arrangement of dietary treatments including the two fat sources (CAN and PKS) and the two additives (without additive, probiotic, and virginamycin) with 5 replicates per treatment. No effect of fat sources or additives on the performance traits and abdominal fat were found. PKS significantly improved triglyceride, high density lipoprotein (HDL) and very low density lipoprotein (VLDL) levels ($P < 0.01$), duodenal villus height and surface area ($P < 0.05$). Likewise the probiotic improved ($P < 0.01$) triglyceride, total cholesterol, and VLDL levels, and increased ($P < 0.01$) both ileal villi height and the ratio of villus height to crypt depth when compared with the nodietary additive group at d 42. On the other hand, virginamycin significantly decreased ($P < 0.01$) total cholesterol and low density lipoprotein (LDL) levels. However, the probiotic interacted positively with PKS leading to increases ($P < 0.01$) in ileal villi height and the ratio of villus height to crypt depth, triglyceride, total cholesterol and VLDL levels at d 42. In conclusion, PKS and the probiotic both exhibited potential improvements for bird health, as indicated by modification of some blood lipid levels and enhancement of some of the morphological parameters in the small intestine. Therefore, the addition of probiotics to fat-supplemented diets can be recommended when the linoleic-oleic group of vegetable oils are used.

Keywords: blood fat, broiler, intestinal structure, *Pediococcus acidilactici*, vegetable oil

S1-0045 Effect of particle size and calcium to non-phytate phosphorus ratio on true calcium digestibility of limestone for broiler chickens

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The purpose of this study was to determine the effect of particle size and calcium (Ca) to non-phytate phosphorus (P) ratio on the true Ca digestibility of limestone for broiler chickens. A limestone sample was obtained from a commercial source, ground and pass through a set of sieves to obtain fine (<0.5 mm) and coarse (1-2 mm) particles. The analysed Ca concentration of both particle sizes was similar (420 g/kg). Six experimental diets were developed using each particle size with Ca: non-phytate P ratios of 1.5:1, 2.0:1 and 2.5:1, with ratios being adjusted by manipulating the dietary Ca concentrations. A Ca-free diet was developed to determine the ileal endogenous Ca losses. Titanium dioxide (3 g/kg) was incorporated in all diets as an indigestible marker. Each experimental diet was randomly allotted to six replicate cages (eight birds per cage) and fed from day 21 to 24 post-hatch. Apparent ileal digestibility of Ca was calculated using the indicator method and corrected for endogenous Ca losses to determine the true Ca digestibility. Ileal endogenous Ca losses were determined to be 127 mg/kg of dry matter intake. Increasing Ca:non-phytate P ratios reduced ($P < 0.05$) the true Ca digestibility of limestone. The true Ca digestibility coefficients of limestone with Ca:non-phytate P ratios of 1.5, 2.0 and 2.5 were 0.65, 0.57 and 0.49, respectively. Particle size of limestone influenced ($P < 0.05$) the Ca digestibility, with the digestibility being higher in coarse particles (0.71 vs. 0.43). In conclusion, the present data demonstrated that increasing dietary Ca concentrations or widening of Ca:non-phytate P ratios lower the true Ca digestibility of limestone in broiler chickens. The true Ca digestibility of coarse limestone particles was higher than those determined for fine particles.

Keywords: calcium, limestone, digestibility, broilers

S1-0046 The substitution of feed additive “Vinivet” based on apicultural products for antibiotic growth promoters in diets for poultry

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The trial conducted in All-Russian Research and Technological Poultry Institute on 3 groups of broiler chicks (Cobb 500, from 1 to 35 days of age) proved the possibility of substitution of feed additive “Vinivet” based on apicultural products for antibiotic growth promoters (AGP) in diets for poultry. The chicks of Group 1 (control) were fed balanced full-diet feed (mash) with no additives; Group 2 was fed the same diet supplemented with 180 ppm of AGP “Stafac” during all 35 days of the trial; experimental Group 3 was fed the same diet as control (without AGP) supplemented with “Vinivet” (5000 ppm of 5 kg per ton of feed). The broilers were housed in cages, 35 birds per treatment. Apicultural products are known for the antiseptic effect on pathogenic gastro-intestinal microbiota, however being inferior to the AGP in antiseptic efficiency. AGP “Stafac” (180 ppm) provided better growth in broilers of Group 2 compared to both control and Group 3. Live bodyweight in Group 2 exceeded control by 2.63; 1.99; 3.22; 0.51 and 2.04% respectively at 6; 14; 21; 28 and 35 days of age. The supplementation with “Vinivet” (5 kg/t) led to the improvements in live bodyweight in Group 3 by 0.61 and 0.86% at 28 and 35 days of age respectively in compare to control. FCR in Group 3 was better by 3.14% compared to control. The analysis of cecal microbiota using T-RFLP method showed substantial decrease in concentrations of pathogenic and opportunistic species in broilers fed “Vinivet”. Concentrations of Staphylococcaceae, Fusobacterium spp., Pertococcaceae and Pasteurellaceae in Group 3 were significantly lower compared to control by 0.91; 0.79; 1.85 and 3.37% respectively; total concentration of cellulolytic bacteria was higher by 7.94%.

Keywords: broilers, antibiotic growth promoters, apicultural products

S1-0047 Lupine in diets for broilers

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The modern cultivars of lupine were studied as a possible substitution of animal-derived protein sources, soybean meal and sunflower cake in diets for broilers. The chicks (Cobb 500 and Cobb Avian 48) were raised from 1 to 36-38 days of age in the Institute's vivarium. The nutritional values of the diets corresponded to the recommendations for these crosses. The unsexed chicks (315 birds per treatment) were housed in cage batteries R-15 (Russia) or Big Dutchman (Germany) under standard management conditions. The substitution of 10 and 15% (on total diet basis) of narrow-leaved lupine (alkaloid content 0.025%) for the sunflower cake was found to improve average live BW in broilers by 1.23 and 0.22%; FCR by 3.57 and 2.38%. To the contrary, the inclusion of 20% of the lupine led to the significant decline in live BW (by 7.64% compared to control). The supplementation of vegetable diets with 15% of lupine instead of soybean meal improved live BW by 0.65% and FCR by 0.59%; the additional supplementation of this lupine-supplemented diet with phytase (100 ppm) improved live BW by 1.80% compared to control. The use of white lupine ("Gamma" cultivar) led to the similar results. The substitution of "Gamma" lupine for sunflower cake and soybean meal (10% of lupine in total diet) improved live BW in broilers by 2.59%. The supplementation of diet with 20% of white lupine with 100 ppm of phytase improved live BW by 1.7% in compare to control; the mixture of phytase and "MEC-SH-4" enzyme preparation (Russia) significantly improved live BW compared to control (by 7.8%, $P < 0.01$) and improved FCR by 10.4%. The digestibility and availabilities of nutrients from experimental diets were similar to control. It was found that the dehulling of lupine allows the production of plant protein source with protein content no less than 40% and fiber content 3.5%. The use of this protein source in broiler diets was found to improve live BW by 5.58-4.57%.

Keywords: broilers, lupine, productivity, enzyme preparations

S1-0048 Individual and combined effects of in ovo injection of creatine monohydrate and glucose on somatic characteristics, energy status and post-hatch performance of broiler embryos and hatchlings

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Two trials were conducted to investigate the individual and combined effects of in ovo injection of creatine monohydrate (CMH) and glucose on the somatic characteristics and energy status of Arbor Acres broiler embryos and hatchlings and the growth performance of chicks during the first week post-hatch. In trial 1, five hundred 17.5-d-old living fertile eggs were randomly assigned to 1 of 5 treatment groups, including 1) a noninjected control, 2) a 0.4 mL diluent-injected control, or 0.4 mL diluents containing 3) 6 mg CMH, 4) 25 mg glucose, or 5) a combination of 6 mg CMH and 25 mg glucose (identified as CMH, G and CMH+G, respectively). In trial 2, after hatch, 48 hatchlings with individual BW close to the average BW of their incubation group were randomly selected and placed in 6 replicates of 8 birds each to evaluate their first week growth performance. Compared with the noninjected and the 0.4-mL diluent-injected control groups, individual injection of CMH or glucose did not affect the hatching time, hatchability, somatic characteristics, or concentrations of glycogen and glucose in the liver and pectoralis major (PM) muscle, although injection of CMH increased concentrations of creatine (Cr) and phosphocreatine (PCr) in the PM muscle on day 19 of incubation (E19.5). However, the CMH+G treatment increased BW relative to set egg weight (SEW) on E19.5, as well as residual yolk sac weight on the day of hatch. Moreover, the CMH+G treatment also increased concentrations of glycogen and glucose in the liver and concentrations of Cr and PCr in the PM muscle on E19.5. Chicks in the CMH+G group also exhibited higher BW gain during the first week than controls. These results indicate that combined injection of CMH and glucose during the last stage of incubation have a synergistic effect on improvement of the energy status of embryos and hatchlings, which is useful for enhancing embryo development, and subsequently improving chick growth during the early stages post-hatch.

Keywords: chick embryo, in ovo injection, creatine monohydrate, glucose

S1-0049 Evaluation of a phytogenic feed additive against an antibiotic growth promoter in broilers challenged with enteric pathogens

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Evaluation of performance, fecal shedding and cecal colonization pattern of certain bacterial species and humoral immune response (HIR) against Newcastle Disease (ND) in broilers supplemented with a phytogenic feed additive (PFA) in comparison to an antibiotic growth promoter (AGP) during infectious stress due to a real pathogen challenge were considered in this study. A 38 d experiment was conducted with 120 male one-day-old Cobb 400 broiler chicks assigned to three treatments, each consisting of 8 replicates. The dietary treatments included a corn-soybean based control diet without added growth promoters and the treatment diets containing either bacitracin methylene disalicylate (BMD, 225 mg/kg) or the PFA (Digestarom® Poultry, 150 mg/kg). Supplementation of broiler diet with PFA improved body weight gain and feed conversion ratio ($P < 0.05$) despite oral challenges with pathogenic *S. enteritidis* and *E. coli* as compared to the control group, the AGP gave an intermediate performance. Fecal *Salmonella* indicated a lower count ($P < 0.05$) at 0 h after challenge in the PFA group compared to the Control. At 24 h after the challenge *Salmonella* and *E. coli* numbers were lower in the AGP and PFA groups than that in the Control ($P < 0.01$). Enumeration of bacteria in the cecal content at the end of the experiment indicated significant reduction of *Salmonella*, *E. coli* and *Clostridium* numbers in the AGP and PFA groups ($P < 0.01$) compared to the Control. However, the number of *Lactobacillus* increased in PFA group in contrast to Control and AGP groups ($P < 0.01$). HIR against ND was identical across the diets at 7 d, increased with age ($P < 0.01$) irrespective of dietary treatments. At 21 d, the ND hemagglutination inhibition (HI) titer was significantly higher in the AGP and PFA supplemented groups compared to the Control. It can be concluded that PFA may serve an effective alternative for enhancing broiler performance especially during periods of infectious stresses.

Keywords: phytogenic feed additive, antibiotic growth promoter, broilers

S1-0050 Effects of Moringa Oleifera seed oil supplemented diet on performance of two genotypes of broiler chickens

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One hundred and twenty (120) day old chicks comprising of two broiler genotypes (Arbor Acre and Ross 308) were subjected to graded levels (0%, 4.5%, 5% and 5.5%) of *Moringa oleifera* seed oil (MOSO) to investigate its effect on their performance (Feed intake, Body weight gain, Feed conversion ratio FCR). The experiment was conducted for 35 days (5 weeks), birds were fed ad libitum and data collected were analysed using a 2x4 factorial design. The experimental diet was subjected to proximate analysis and results showed that crude protein content was inversely proportional while fat content was directly proportional to MOSO inclusion in the diet. When the diet supplemented with graded level of MOSO were fed to the chickens, significant ($P < 0.05$) differences in the feed intake were observed within each genotype from weeks 1- 5 with the values decreasing as the level of MOSO inclusion increased. When the interaction between diets and genotype were observed a significant ($P < 0.05$) effect of both factors were recorded at week 1, 3 and 5 with Arbor acre showing reduced intake on diet containing 5.5% MOSO at week 1 and 3 and the Ross 308 showing reduced intake on the same diet at week 5. Interaction of both factors had no significant ($P > 0.05$) effect on body weight gain values, and both genotype had better body weight gain (10.04%) at 4.5% MOSO inclusion level. The interaction of both factors (Diet and Genotype) showed best FCR for diet 1 and 3 at weeks 1, 2, 4 and 5 in Ross 308 strain, while Arbor Acre showed best value at week 3 for diet containing 5.5% MOSO. The results showed that 4.5% MOSO inclusion contributed significantly to body weight gain in the two broiler genotype at 35-day. Also, the two broiler types respond to MOSO supplemented diet at different rate.

Keywords: *Moringa oleifera* seed oil, broiler, genotype, feed intake, body weight gain.

S1-0052 A prebiotic in broiler diets

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Probiotics, prebiotics, symbiotics, synbiotics, phytobiotics are widely used at present as a substitution for antibiotic growth stimulators. The most prevalent are probiotic preparations while prebiotics – substrates stimulating natural microbial population of gastro-intestinal tract – are still lesser scrutinized. The aim of the study presented was the evaluation of efficiency of a prebiotic preparation in diets for broiler chicks. The preparation was obtained by mechano-enzymatic hydrolysis of dried biomass of *Saccharomyces cerevisiae* yeast and contained active mannanoligosaccharides (non less than 4%), β -glucanases (up to 20%), protein (45%) including amino acids (20%), B vitamins (up to 25 ppm). The trial was conducted on cage-housed Cobb 500 broilers during 1-37 days of age, 35 birds per treatment. The prebiotic preparation (1000 ppm during all the trial) was found to improve live BW by 2.9% and FCR by 2.9%. The digestibility of dry matter in experimental group was better by 4.1%, protein by 3.1%, fat by 4.2%, fiber by 1.1%, nitrogen by 3.8%. T-RFLP analysis of cecal microbiota revealed higher concentration in experimental group of “healthy” bacilli (*Bacillales*) and bifidobacteria (*Bifidobacteriaceae*) with high antagonistic activity against pathogens and cellulolytic activity. In experimental group higher concentrations of “useful” saccharolytic, cellulolytic, and lactate-fermenting bacteria (*Eu-bacterium*, *Veillonella*, *Ruminococci*, *Bacteroides*) and lower concentrations of pathogens (*Pasteurellas*, *Actinomycetales*, *Staphylococci*, *Campylobacteria*, *Mycoplasmas*, *Enterobacteria*, *Fusobacteria*) were also found. Histological examination showed the improvements in histoarchitectonics of the small intestine in broilers fed the prebiotic preparation.

Keywords: prebiotic, microbiota, broilers, histology

S1-0053 Non-traditional legumes in diets for broilers

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Legumes with the exception of soybeans are still considered “non-traditional” components of commercial diets for poultry. Though these crops contain high amounts of protein and amino acids they also contain inhibitors of proteolytic enzymes, alkaloids, tannins and other anti-nutritional factors affecting productivity in poultry. The study presented was aimed at the evaluation of efficiency of lupine (alkaloid content 0.045% ; Trial 1) and horse beans (*Vicia faba* L.; tannins content 1.28% , Trial 2) in diets for broiler chicks. Broilers (Cobb Avian - 48, 35 birds per treatment) were raised to 38 (Trial 1) and 36 (Trial 2) days of age. In Trial 1 broilers were fed 10, 15 and 20% of lupine respectively to age periods of 5-14; 15-28 and 29-38 days; the diets included multi-enzyme preparation Multifabazyme (complex of protease, xylanase, glucanase, pectin-lase, galactosidase activities) in doses 50, 75 and 100 ppm respectively to the 3 age periods. Control was fed 5% of lupine. In experimental group the improvements in live BW by 6.1% and FCR by 3.2% due to the improvements in digestibility of DM (by 2.7%), protein (by 2.7%), fat (by 1.8%), fiber (by 3.2%) and nitrogen (by 3.7%) were found. The biochemical analysis of blood serum proved the beneficial effect of this enzyme preparation on the metabolism (anabolic processes, lipid exchange, calcium and phosphorus supply). In Trial 2 broilers were fed 12.5 and 15% of horse beans respectively to age periods of 5-28 and 29-36 days; both these diets contained 75 ppm of enzyme preparation Cellolux-F (complex of cellulase, xylanase and glucanase activities). Control group was fed no beans. In experimental group the improvements in live BW by 3.6% and FCR by 5.7% due to the improvements in digestibility of DM (by 1.6%), protein (by 1.5%), fat (by 3.2%), and nitrogen (by 2.0%) were found.

Keywords: lupine, horse beans, broilers, enzyme preparations, productivity

S1- 0054 Morphological changes in immune related organs, humoral and cell mediated immune response and intraepithelial lymphocytes evaluation of broiler chickens fed *Bacillus subtilis* or Zinc Bacitracin

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The present research was conducted with the aim is to analyze effect of dietary supplementation of *Bacillus subtilis* (B. subtilis) on humoral and cellular immune responses, relative weights of lymphoid organs and intraepithelial lymphocytes (IEL) count in broilers. Total 120, day-old broiler chicks, reared in environmentally controlled sheds were divided into four groups (Negative Control, NC; Positive Control, PC; BS- 0.05; BS- 0.1) maintained on corn-soy based basal diet or basal diet supplemented with 10% Zinc Bacitracin, 0.05g/kg or 0.1g/kg at rate of 2.0×10^{10} cfu/g B. subtilis respectively. Each group had 30 birds with three replicates (n=10) each. Differences were considered significant at $P < 0.05$. Birds were slaughtered on 21st and 35th day, and blood and organs (liver, spleen, thymus and bursa of Fabricius) were collected. Treatment groups displayed higher antibody titer against ND and sheep RBC (SRBCs) compared to NC at day-35th. Significant cell mediated immune response post Phytohemagglutinin-P injection was attained by BS-0.1 group at 24hr and both BS- 0.1 and BS- 0.05 at 48 and 72hrs compared to PC and NC, respectively. Spleen weight was found significantly higher in all the treatment groups at day-21st and liver, spleen, thymus weights, respectively compared to NC at day- 35th. BS-0.1 gain higher relative bursal weight at day- 21st. The IEL population in the ileal mucosa was found significantly higher in group BS-0.1 than group PC and NC on Day-35. BS-0.05 was also significantly different from the NC group in mucosal infiltration of lymphocytes on day-35. In conclusion, the B. subtilis type probiotics displayed better cellular and humoral immunity, as well as relatively greater weight of lymphoid organs in healthy birds reared under control environment, which suggests that this probiotic may be a proper substitute of antibiotic.

Keywords: broiler chicken, bacillus subtilis, cellular and humoral immunity, organs morphology, IEL

S1-0055 Effects of various levels of l-carnitine on blood biochemical parameters in female broiler chickens at 10 days of age

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This study was conducted to investigate the effects of various level of L-Carnitine (100, 200, and 300 mg/kg in diets) in diets containing 3% soybean oil on blood biochemical parameters in female broiler chickens. A total of 240 one-day old female broiler chicken (Ross 308) were randomly allocated into 4 dietary treatments of 3 replicates with 20 birds in each by a completely randomized design. Hematological and Blood biochemical parameters of female broiler chickens were tested at 10 days of age. The results of present study have shown that SGOT, SGPT, and albumin were significantly increased by increasing of L-Carnitine levels during 10 days of age ($P < 0.05$). The triglyceride was significantly reduced by increasing of L-Carnitine levels ($P < 0.05$). However, numerical raises in blood LDL-cholesterol and LDL as well as a reduction in Mg and glucose was observed by increasing of L-Carnitine levels. The results of present study have shown that red blood cells and MCV were significantly increased by increasing of L-Carnitine levels during 10 days of age ($P < 0.05$). In addition, leukocyte percentage influenced by increasing of L- Carnitine levels ($P < 0.05$), although total white blood cell had un-significant changes. Treatment effects were un-significant on other parameters (Hb, MCH, MCT, and MCHC) at 10 days of age.

Keywords: L- Carnitine, starter, blood parameters, broiler, WPC

S1-0056 Effects of various levels of l-carnitine on performance and carcass characteristics of female broiler chickens from 1 to 10 days of age

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This study was conducted to investigate the effects of various level of L-Carnitine (100, 200, and 300 mg/kg in diets) in diets containing 3% soybean oil on performance and carcass yield characteristics in female broiler chickens. A total of 240 one-day old female broiler chicken (Ross 308) were randomly allocated into 4 dietary treatments of 3 replicates with 20 birds in each by a completely randomized design. Performance and carcass yield characteristics of female broiler chickens were tested at 10 days of age. The results of present study have shown that the weight of live body, carcass, breast and yolk sac were significantly decreased by increasing of L-Carnitine levels during 10 days of age ($P<0.05$). Increasing of L-Carnitine levels had no significantly effects on weight of liver, gizzard, proventriculus, and heart. The result of this study showed that the supplementation of diet with L-Carnitine had negative effect on body weight and carcass weight up to 10 days of age.

Keywords: L-Carnitine, broiler, 10 days, performance, Yulk Sac

S1-0057 Effects of *Neurospora crassa* fermented palm kernel cake utilization in the diet on the egg yolk cholesterol and fat contents as well as egg yolk color index of poultry

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Two experiments had been conducted to determine the effects of *Neurospora crassa* fermented palm kernel cake (PKCF) utilization in the diet on the egg yolk cholesterol and fat contents as well as egg yolk color index of poultry. Experiment 1 employed 300 72-week old Arab laying-hens that were assigned to experimental diets (A=0% PKCF, B=7.25% PKCF, C=10.15% PKCF, and D=13.05% PKCF). Experiment 1 was performed in a completely randomized design with four treatments (experimental diets) and five replicates. Experiment 2 employed 240 local Kamang laying-ducks with two groups of age (18 month old and 6 month old). Experiment 2 was performed in a randomized complete block design with four treatments and two blocks as replicates. Treatments were level of PKCF (A=0% PKCF, B=5% PKCF, C=10% PKCF, and D=15% PKCF) in diets. Results of experiment 1 indicated that the utilization of PKCF in diets very significantly reduced ($P<0.01$) the egg yolk cholesterol and fat contents of Arab laying-hens. However, the egg yolk color index was not influenced ($P>0.05$). Results of experiment 2 also indicated that the utilization of PKCF in diets very significantly declined ($P<0.01$) the egg yolk cholesterol and fat contents of local Kamang laying-ducks. The egg yolk color index was very significantly influenced ($P<0.01$) by diets. In conclusion the utilization of PKCF in diets decreased the egg yolk cholesterol and fat contents, and improved the egg yolk color index of poultry.

Keywords: palm kernel cake, cholesterol, fat, color index, egg yolk

S1-0058 Response of laying hens to natural sources of vitamin premix

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Two hundred and forty (240) Harco-black laying hens were used in an 8-week feeding trial to evaluate the effects of naturally produced vitamin premix on bird performance and egg quality characteristics. The birds were randomly allotted to four treatment diets of sixty (60) birds in the form: Control (no Vitamin Premix), 0.5% Commercial Vitamin Premix (CVP), 0.3% Natural Vitamin Premix (NVP) and 0.6% Natural Vitamin Premix (NVP). NVP was formulated as a cocktail of herbs, spices and extracts. Data on Body weight gain (BWG), Average Daily feed Intake (ADFI), Hen-Day Production (HDP), Feed Conversion Efficiency (FCE) and Egg quality characteristics were measured and recorded. The result showed significant differences ($P < 0.05$) in laying performance across the treatments. Higher ($P < 0.05$) HDP, ADFI, and FCE were observed in hens fed 0.3% NVP supplemented diet (86.69%, N98.07, and 114.04g/b/d respectively). Weekly body weight gain was not affected ($P > 0.05$) by the dietary intervention. External and Internal egg characteristics were not affected ($P > 0.05$) by the treatments. Higher yolk colour score ($P < 0.05$) was observed in eggs from hens fed 0.6% NVP supplemented diet (5.05), followed by 0.3% NVP (4.13). Control and CVP supplemented diet recorded similar ($P > 0.05$) yolk colour scores (≈ 3.1). The study suggest a relative improvement in laying performance for hens fed diets containing 0.3% NVP.

Keywords: laying hens, vitamin premix, egg yolk colour

S1-0060 Graded, pre- and post-pelleting additions of whole wheat influences relative gizzard weight, feed conversion efficiency and energy utilisation in broiler chickens

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The strategy of whole grain feeding is meeting increasing acceptance and various approaches may be adopted in pursuit of this strategy. In this study, a ground wheat-based (600 g/kg) control diet was compared with graded whole wheat additions (7.5, 15.0, 30.0%) incorporated into the ration either pre- or post-pelleting. The seven dietary treatments were offered to eight replicates (6 birds/cage) or a total of 336 male Ross 308 broiler chickens from 7 to 28 days post-hatch. A range of growth performance and nutrient utilisation parameters were determined by standard methods. Pre-pelleting whole grain additions numerically increased relative gizzard weights by up to 13.0% (16.44 versus 14.55 g/kg) at the 30% inclusion. However, post-pelleting additions significantly increased gizzard weights by up to 37.5% (20.01 versus 14.55 g/kg). Overall, whole grain feeding improved FCR ($P < 0.03$) by 4.35% (1.441 versus 1.506) and at 30%, pre-pelleting whole grain additions significantly improved AME, ME:GE ratios and AMEn but not at lower whole grain additions. In contrast, all three post-pelleting whole grain additions significantly enhanced nutrient utilisation with average improvements of 0.82 MJ in AME (12.89 versus 12.07 MJ/kg), 6.10% in ME:GE ratios (0.743 versus 0.700), 7.36 % units in N retention (66.15 versus 58.79%) and 0.68 MJ in AMEn (11.70 versus 11.02 MJ/kg). Interestingly, there was a quadratic relationship between relative gizzard weights and AME ($r = 0.650$; $P < 0.001$) where the regression equation was as follows: $AME(MJ/kg) = 3.190 + 0.936 * \text{gizzard weight}(g/kg) - 0.022 * (\text{gizzard weight}(g/kg))^2$. In this study the optimal AME of 13.15 MJ/kg, would be generated by a relative gizzard weight of 21.27 g/kg. Thus it appears that AME improvements will plateau at a given gizzard weight increase and further enlargement of the gizzard will not benefit energy utilisation. Further research should be conducted to identify how best to take advantage of whole grain feeding regimes.

Keywords: apparent metabolisable energy, relative gizzard weights, whole grain feeding

S1- 0061 The effect of durian fruit waste fermented by phanerochaete chrysosporium and Neurospora crassa in the diet on laying hen performance and egg quality

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An experiment was conducted to determine the effect of fermented durian fruit waste (*Durio zibethinus*) with *Phanerochaete chrysosporium* and *Neurospora crassa* on production performances and egg quality of laying hen. This experiment was arranged in a completely randomized design with five dietary treatments: 0%, 10%, 20%, 30% and 40% fermented durian fruit waste with *Phanerochaete chrysosporium* and *Neurospora crassa* in the diets and four replications. 200 laying hen Isa Brown (22 week of age) were randomly allocated into 5 treatments (10 hens per treatment). Variable measured were feed intake, egg production, feed conversion, egg mass, egg weight, egg cholesterol and egg yolk colour. Results of the experiment indicated that feed intake, egg production, egg mass, egg weight, feed conversion not affected but egg cholesterol and egg yolk colour were affected ($P < 0.01$) by increasing fermented durian fruit waste with *Phanerochaete chrysosporium* and *Neurospora crassa* in the diet of laying hen. Egg yolk colour in E treatment (40% fermented durian fruit waste with *Phanerochaete chrysosporium* and *Neurospora crassa*) was the highest than other treatment, but the lowest on egg cholesterol. The conclusion of the experiment that up to 40% fermented durian fruit waste with *Phanerochaete chrysosporium* and *Neurospora crassa* maintained production performances, reduced egg cholesterol 22.16% and increased egg yolk colour 20.61%.

Keywords: egg quality, fermented durian fruit waste, layer performance, *Neurospora crassa*, *Phanerochaete chrysosporium*

S1- 0063 Impact of in ova injection of some water soluble vitamins on post-hatch broilers performance

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The aim of this study was to evaluate the impact of in ova injection on post-hatch growth performance and carcass criteria of Hubbard broiler chicks. A total of 250 eggs were equally divided into five groups. The first group served as negative control (without injection), the second group was considered as positive control (injected with 0.5 ml sterilized distilled water), the third, fourth and fifth groups were injected with 1 mg B2, 0.5 mg B6 or 100 mcg Folic acid solved in 0.5 ml sterilized distilled water; respectively. The injection was done at the 17th day of incubation period. The hatched chicks from each group were divided into two replicates and were kept till 6th week of age. They were subjected to similar management conditions and fed starter, grower and finisher diets for two weeks each. During this period, body weight (BW) and weight gain (WG), feed consumption (FC) and efficiency (FCR) were weekly evaluated. At 42 days of age, 6 birds from each treatment were sacrificed to evaluate different carcass criteria. The results revealed significant differences between treatments till 5th week of age for BW and till 3rd week for WG but not significant for FC or FCR. In ova injection with B2 showed the highest significant positive impact on chicks BW till 5th week of age and on WG till 3rd week; however, B6 and folic acid injection had a positive effect on both BW and WG till the 2nd week of age only. Different carcass criteria didn't show any significant differences between the treatments and their control groups; however, breast weight and percentage of the chicks from B2 treatment were numerically higher than those of the control groups (624 vs.583 and 589 g and 34.4 vs. 33.6 and 33%; respectively). Therefore, it may be concluded that in ova injection with B2 is in favor of BW if the broilers supposed to be marketed at 35 days of age. In-ova injection of B6 or folic acid may have advantage on broilers' performance throughout the starter period only.

Keywords: in ova injection, water-soluble vitamins, broilers performance

S1-0064 Performance and intestinal microbial profile of broiler chickens supplemented with a blend of protected organic acids and essential oils

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The increasing concern about the use of antibiotics in poultry production has changed the ways in which producers manage the birds' overall health. Currently, additives with anti-microbial and growth promoting effects are added in poultry feeds to prevent and control GI-tract infections that adversely affect performance. A study was conducted to determine the effects of a blend of protected organic acids (OAs) and essential oils (EOs) in performance and intestinal microbial profile of broiler chickens. A total of 612 Ross 308 day old chicks were randomly assigned to receive 1 of 3 treatments for 28 d: 1) basal diet with no antibiotic + 100 ppm lasalocid (T1) (n=204), 2) T1 + 300 ppm of protected OAs and EOs (T2) (n=204), and 3) T1 + 1500 ppm of protected OAs and EOs (T3) (n=204). A completely randomized design with 3 treatments, 12 replicates, and 17 birds in each replicate was used. On d 14 and 28, 1 bird from each pen was sacrificed to collect ileal and cecal samples for microflora analysis using high-throughput sequencing based on 16S rRNA genes. The BW of birds in T2 and T3 at d 21 was significantly increased relative to T1 ($P<0.02$, 4.6%), as was the BW of birds in T2 at d 28 ($P<0.05$, 2%). The FCR was not different between treatments; however, there was a trend towards improved FCR at d 21 in T2 ($P<0.09$, 5.5%) and T3 ($P<0.06$, 5.6%), as well as at d 28 in T2 ($P<0.06$, 5.7%). Sequencing data at d 14 and 28 revealed retained complexity and overall structure of the ileal and cecal microbiota across treatments. However, the intestinal microbial profile of treatments changed in between these time points. Compared to T1, significant changes in the abundance of some *Lactobacillus* species within the cecum of birds in T2 and T3 were found at d 28. Overall, the supplementation of a blend of protected OAs and EOs had no adverse effect on the microbial diversity of the intestine and appears to offer benefits with respect to gut health and productivity in broiler chickens.

Keywords: organic acids, essential oils, performance, microflora, broilers

S1- 0065 Raw and autoclaved rice bran inclusion in broiler chicken diets on performance and bone mineralization

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Increasing inclusion levels of raw rice bran (RRB) or autoclaved rice bran (ARB) was studied on tibia characteristics, nutrient digestibility, microflora population, and broiler chicken performance. A total of 420 male broiler chickens (Ross 308) was randomly assigned to 7 diets with 3 replicates of 20 chickens in each at a completely randomized design. Diets were designed as basal diet (without rice bran (RB) addition), and basal diet plus RRB or ARB at the levels of 6, 12, or 18% for grower (15-28 days of age) or finisher (29-42 days of age) periods. The feed was offered ad libitum. All antinutritional parameters of RB were reduced by autoclaving. Independent comparisons showed significant decreases in feed intake and feed conversion ratio between birds fed diets contained RB in contrast to control at all ages ($P<0.05$). Such diets led to significantly decreases in digestibility of dry matter, organic matter, ether extract, crude protein, and gross energy as well as decreases in *Escherichia coli* population in distal intestinal ($P<0.05$). Moreover, *Escherichia coli* population significantly decreased by the incorporation of RRB in comparison of ARB at 42 days of age ($P<0.05$). The highest lactic acid bacteria were found by the incorporation of 12 or 18% RRB in diets ($P<0.05$). The lowest tibia weight was obtained by incorporation 18% ARB in diets ($P<0.05$). The use of RB in diets rather control result in significant reduction in cholesterol and high-density lipoproteins contents ($P<0.05$). The results have shown that autoclaving have positive effect on nutrition values and omit of antinutritional factors of RB, but inclusion of RB had no improvement on broiler performance. In addition, the high levels (18%) of RRB and ARB reduce bone ossification.

Keywords: : autoclaving rice bran, broiler performance, digestibility, microflora, tibia

S1-0066 Effects of 1 α -hydroxycholecalciferol 1 α - OHD3 on tibia bone traits and mineral retention in broilers

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In this research, the effects of dietary supplementation and in ovo injection of 1 α -OHD3 in a low-Ca and Low-P diets on broiler growth performance, tibia physical and chemical traits, blood serum parameters and mineral retention were studied. Four hundred male broiler chickens were randomly allotted to 4 dietary treatments with 5 replicates of 20 birds each and grown over a 28-d experimental period. The treatments were: T1-positive control (100% Ca and P Ross recommendation), T2-negative control (50% Ca and P Ross recommendation), T3: negative control + in ovo injection of 1 α -OHD3, and T4: negative control + feeding of 1 α -OHD3. The diets were offered ad libitum throughout the experiment. The experiment was approved by the Animal Ethics Committee of the Tarbiat Modares University. Data were analyzed using the General Linear Model procedure of SAS appropriate for a completely randomized design. Treatment means were compared using the Duncan's Multiple Range Test, and values were considered statistically different at $P < 0.05$. In comparison to positive control, feeding of low Ca and P diets decreased body weight. In ovo injection and feeding of 1 α -OHD3 did not improved body weight gain of broiler chickens. Feed efficiency, abdominal content and pancreas weight was not affected by treatment groups. The weight of bone but not bones length was affected by treatments. Bone weight was lower in negative control but was not different between positive and T3 and T4 (negative + in ovo and feeding of 1 α -OHD3). Bone ash, calcium and phosphorous was higher in birds receiving 1 α -OHD3 as feed. Treatments did not effect zinc and manganese content of bones. It could be that the in ovo injection and feeding of 1 α -OHD3 though did not affect broilers performances, it resulted to stronger bone characteristics.

Keywords: 1 α -OHD3, broiler, in ovo injection, retention, tibia

S1-0067 Effect of *Jatropha gossypifolia*(JG) leaves extract and honey application through drinking water on performance and protein metabolism in broilers

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Medicinal plants may act as beneficial feed and water additives in livestock, especially poultry. *Jatropha gossypifolia* (JG) popularly known as bellache bush contains a variety of active substances with a wide range of pharmacological activities such as nimbin, nimbolite, quercetin, alkaloids, phenolic compound, flavonoid, terpenoids and essential oil. The constituents have immunomodulatory properties. The objective of this study was to determine the effects of application of JG supplemented with honey through drinking water on broiler performance, health status and protein metabolism. A total of 75 day old broilers (abor acre) of mixed sexes were randomly divided into five groups. JG leaves extract were applied through drinking water (3 days application, 6 days interval) and were applied at 0.10 ml/kg and 0.20ml/kg body weight on two groups per treatment. One of the group was supplemented with honey and one control groups. Effect of JG supplementation on performance, health and blood protein were determined. After four weeks of treatment with different experimental diets, blood samples were collected from the birds for biochemical analysis. The results showed no harmful effect on health and performance of broilers given JG leaves extract. There was little improvement on the feed conversion ratio of birds given JG with honey. There were significant differences ($P < 0.05$) in total serum protein and Albumin with the bird on 0.20ml/kg with honey having the highest values of 6.25 and 3.39g/dl respectively. The results showed no significant difference in liver biomarkers (AST, ALT) ($P > 0.05$) and reduction in kidney biomarkers (urea, creatinine) of birds given JG. Serum globulin contents were significantly higher ($P < 0.05$) in JG treated group as compared to control. This might be due to the ability of JG to increase immunoglobulin concentration. In conclusion, further research is required to identify the mechanism and mode of action of active ingredients of JG extracts.

Keywords: *Jatropha gossypifolia*, serum, protein, immunoglobulin, medicinal

S1-0068 Effect of resistant starch on growth performance and dietary calcium and phosphorus absorption in broiler chicks

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Fermentation products of resistant starch (RS) such as butyrate and propionate lower large intestinal lumen pH, increasing solubility and absorption of minerals. Against this backdrop the effect of four RS products; raw green banana starch (RGS, 76.81% RS), raw potato starch (RPS, 43.49% RS), hydrothermally modified green banana starch (HMGBS, 31.68% RS) and hydrothermally modified potato starch (HMPS, 34.7% RS) on absorption of dietary calcium, Ca and phosphorus, P and growth performance of broiler chicks from 8-28d were compared. One hundred and twenty-eight, 8d-old birds were randomly allocated to 4 dietary treatments with 4 replicates and 8 birds per replicate, housed in battery brooder cages. Broiler starter diets containing 43-49% starch with 5% RS supplied by each RS product were formulated. The effect of the RS products on performance, tibia ash as well as serum Ca and P 0-8h after an oral administration of 125 mg Ca (as calcium citrate) was assessed. Performance variables; weight gain, feed intake were monitored and feed conversion ratio (FCR) estimated. Curves for serum Ca and P over 8 h were plotted and area under the curve (AUC) estimated by the trapezoidal method. Data were analysed using ANOVA and means separated using DMRT. Performance variables were unaffected by RS source except for FCR at 15-22d which differed ($P<0.05$) for RPS and HMPS (1.67 vs 1.44). Similarly, tibia ash was lowest ($P<0.05$) for birds on the RPS diet (39.91%) and highest for the HMGBS diet (46.79%). AUC for serum Ca varied significantly ($P<0.05$) for HMGBS and HMPS at 0-2h (18.49 mg.dL⁻¹.h⁻¹ vs 13.28 mg.dL⁻¹.h⁻¹) and 0-6h (24.54 mg.dL⁻¹.h⁻¹ vs 15.59 mg.dL⁻¹.h⁻¹) and between HMGBS and RPS, HMPS at 0-8h (72.22 mg.dL⁻¹.h⁻¹ vs 41.29 and 45.46mg.dL⁻¹.h⁻¹). AUC for serum P was unaffected by RS type. HMGBS significantly modified serum Ca compared to RPS, but was comparable to HMPS and RGS. Higher inclusions of RS in the diet may result in more visible effects on variables measured.

Keywords: hydrothermal modification, resistant starch, broiler chicks, performance

S1-0069 Investigation of the effect of different levels of probiotic and physical form of feed on small intestinal morphology in Japanese quail

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The aim of this study was to investigate the effect of different levels of probiotic and physical form of feed on small intestinal morphology in Japanese quail. Thus, 240 Japanese quail chickens were used in a factorial experiment with completely randomized design with 6 treatments, 4 replicate and 10 chickens in each replicate for 42 days. The experimental treatments were, Mash feed without probiotic in water, Mash feed with 1% probiotic in water, Mash feed with 2% probiotic in water, Crumble feed without probiotic in water, Crumble feed with 1% probiotic in water, Crumble feed with 2% probiotic in water. The experimental diets were formulated based on national research council and UFFDA software. In 42 days of age, after weighing all of birds, one bird from each pen was slaughtered and relative weight and length of different parts of intestine and morphologic characteristics (villi length and width and crypt depth) were studied. Data were analyzed with GLM procedure using SAS 9.1. Means were compared via least square means and Tukey test ($P<0.05$). The results of small intestinal morphology of Japanese quail chickens showed that villi length and width and crypt depth of Japanese quail chickens were significantly affected by treatments ($P<0.05$). Chickens fed with crumble feed with 2% probiotic in water had the highest villi length and crypt depth. The results showed that relative weight of different compositions of gastrointestinal tract of quails were significantly affected by the experimental treatments ($P<0.05$). Also, the experimental treatments had significant effect on relative length of duodenum and cecal ($P<0.05$). According to the results, the protexin probiotic had significant effect on small intestinal morphology improvement and relative length and weight of different parts of small intestine improvement that resulted in enhanced nutrients digestibility and finally improvement in feed efficiency and average daily gain in Japanese quail chickens.

Keywords: protexin probiotic, feed physical form, small intestinal morphology, Japanese quail

S1-0070 Dietary methionine supplementation alters serous biochemical indices, antioxidant capacity, immunity parameters and endocrine hormones in Xinyang Green Shell laying hens

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Primary objective of the experiment was to investigate impacts of dietary methionine on physiological variation in Xinyang Green Shell laying hens (34~43 weeks). A total of 1 440 hens were randomly allocated into 6 groups with 4 replicates of 60 birds each. A basal corn-soybean meal diet with 0.24% methionine was given to the control group. The methionine supplementation dietary with 0.27%, 0.30%, 0.33%, 0.36%, and 0.39% concentration in diets respectively, were applied in the experimental groups. The trial was consisted of one week for acclimatization and 10 weeks for testing. In the end, 48 layers (2 birds from each replicate) underwent 24 h fasting were sacrificed for sample collection. The results indicated methionine supplementation affected biochemical levels of TG and T-CHO, antioxidant activity of MDA and GSH-Px, immune capacity of IgG, IgM and C4 in serum significantly. However, no significant differences were observed among endocrine hormones parameters. Compared with the control group, levels of TG in 0.36% and 0.39% groups increased 20.05% and 26.41%; levels of T-CHO in 0.27% and 0.30% groups decreased 31.46% and 17.45%; levels of MDA in 0.27%, 0.30%, 0.33%, 0.36% groups diminished 19.44%, 17.28%, 9.47% and 6.98%, respectively; activities of GSH-Px in 0.27% and 0.30% groups elevated 4.15% and 3.71%, and levels of IgM in 0.27% and 0.30% groups improved 14.62% and 11.65%. In conclusion, dietary methionine contributes to improving systemic homeostasis by upregulate the lipometabolism, immune function and antioxidant status in laying hens.

Keywords: methionine, laying hens, biochemical indices, antioxidant capacity, immune parameters

S1-0071 Aflatoxin levels in poultry feed and its feeding effect on productive and reproductive performance of three native genotypes of Bangladeshi chickens

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Aflatoxin (AF) is the most commonly known mycotoxin in poultry resulting adverse effects. It is generally affect the liver and/ the kidney and causing significant economic losses. The objectives was to estimate AF level in poultry feed and find out the changes on productive and reproductive parameters in laying hens during AF ingestion. Considering storage duration, 360 poultry feed samples were measured from 18 different locations by using VERATOX- a quantitative AF test kit covering the period of June to August' 2015. Since then, 120 laying hens (16 wks old) from three native genotypes of Bangladeshi chickens were randomly allocated into 4 different dietary treatment groups (0.0, 0.5, 1.0 and 1.5 mg of AF/kg of feed) having isonitric and isocaloric basal feed. The AF (B1, B2, G1, and G2) was obtained from *Aspergillus flavus* and *Aspergillus parasiticus* grown in corn grains; the grain was sterilized, grounded and added to basal diets to achieve desired AF levels. Contaminated diets were supplied to the hen up to 40 weeks of ages. Data were analyzed following 3×4 factorial arrangement. Results has indicated that poultry feed are prone to AF contamination in these areas and it increased with the increasing duration length of storage. Major productive and reproductive parameters at 40 weeks of age were studied. The fertility, hatchability and embryonic mortality were significantly different ($P<0.05$) among genotypes and treatments. No significant difference ($P>0.05$) was observed in age at sexual maturity, dead in germ and abnormal chicks. Total egg production and body weight at 40 weeks of age were significantly difference ($P<0.01$) among genotypes and treatments but no significant difference ($P>0.05$) in survivability and egg weight. These results suggest that AF chronic ingestion affects productive and reproductive performance of laying hens. Besides, higher AF levels in feed than the permissible level that might be risk for poultry industry.

Keywords: Aflatoxin, poultry feed, performance, native genotypes, Bangladesh.

S1-0072 Effects of garlic powder, vitamin E and antioxidant supplementation on broiler performance, carcass analysis and oxidative stability of meat

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Two experiments were conducted to determine the AME content of garlic powder, and to investigate the effect of it as well as vitamin E and Loxidan (as a commercial antioxidant) supplemented in diets on growth performance, carcass analysis and meat oxidative stability. In the first experiment, the AME of the garlic powder was determined in Leghorn roosters fed diets containing four garlic powder levels including 5, 10, 15 and 20% of it. In the second experiment, a 2*2 factorial arrangement including two levels of garlic powder (0 and 2%), two levels of additional vitamin E (0 and 200 mg/kg), and 2 levels of antioxidant (0 and 200 mg/kg) were employed using four replicates and twelve broiler chicks in each. The AME turned out to be 2370, 2300, 2090 and 2165 kcal/kg for diets containing 5 and 10, 15 and 20% garlic powder, respectively. Moreover, growth performance, the ratio of eviscerated carcass, breast, thigh and abdominal fat to live weight were not affected by diets. However, applying garlic powder together with antioxidant resulted in better daily weight gain and eviscerated carcass. TBA and pH of the meat stored in refrigerator for six days, appeared to have higher values, compared to the fresh and meat kept at refrigerating temperature and garlic exacerbated the situation ($P<0.05$), but freezing temperature did not affect these indices. The results indicate that simultaneous dietary supplementation of garlic powder, vitamin E and antioxidant have no effect on growth performance, but it improves the oxidative stability of meat. In addition, applying vitamin E and antioxidant in diets can result in better meat oxidative stability ($P<0.05$). It can be concluded that supplementing diets with natural or synthetic antioxidant substance can improve the quality of meat during storage, but, there is no considerable influence on growth parameters.

Keywords: broiler performance, meat quality garlic, vitamin E, antioxidant

S1-0073 Influence of supplementing graded levels of arginine to diets based on canola meal untreated or treated with copper on performance, organ weights and some blood metabolites of broilers

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This experiment was conducted to study the effects of copper (Cu) ions treatment of canola meal (0, 125 and 250 mg/kg) and different levels supplemental arginine (0, 0.1 and 0.2% Arg) on performance, organ weights and some blood metabolites in broilers. During a three week period of experiment (22- 42d), a total of 405 male broilers were used in completely randomized design with a 3×3 factorial experiment with 9 treatments, 5 replicates and 9 birds in each replicate. Feed intake was not affected ($P>0.05$) by treatments during period of experiment. Average daily gain and feed conversion ratio were improved ($P<0.05$) by 250 mg/kg Cu treatment. Canola meal treatments with Cu ($P<0.01$) and added 0.2% Arg ($P<0.05$) significantly increased carcass yield and proportion of breast muscle. Relative weight of thigh was also significantly ($P<0.05$) increased by 250 mg/kg Cu treatment and 0.2 % arginine supplementation. The use of 0.2% Arg significantly reduced proportion of abdominal fat ($P<0.01$) and lungs weight ($P<0.05$) and conversely increased proportion of duodenum ($P<0.05$) and jejunum ($P<0.01$). Treatment of canola meal with Cu declined thyroid glands weight ($P<0.01$) and increase T3 ($P<0.05$) and T4 ($P<0.01$) plasma levels. Addition of 0.2 % Arg significantly declined ($P<0.05$) plasma glucose concentration. Moreover, the uric acid concentration was also decreased ($P<0.01$) by using supplemental Arg. However, plasma concentrations of cholesterol, high density lipoprotein, triglycerides, aspartate amino transferase, alanine amino transferase, gamma- glutamyl transferase, lactate dehydrogenase, alkaline phosphatase and creatine kinase were unaffected ($P<0.05$) by dietary treatments. In conclusion, the results of this study show that treatments of canola meal with Cu could alleviate adverse effects of glucosinolate on broilers performance. Moreover, the results of this study indicate that no effects of added supplemental Arg up to 0.2 % on the growth performance and thyroid status.

Keywords: arginine supplementation, Cu treatment, performance, broilers

S1- 0074 Passage rate of digesta from soybean meal, wheat bran and rice bran diets with or without a multi- enzyme supplementation in broiler chickens

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Two hundred and sixty 3-week-old Arbor Acres broiler chickens were used to determine the effect of a multi-enzyme (Biomix) supplementation on the rate of feed passage, mean retention time, gut morphology, digesta viscosity and performance in the birds. Of the 6 diets containing soybean meal, wheat bran and rice bran, 3 were supplemented with the enzyme and 3 without enzyme. Each diet had 6 replicates with 6 birds each. Cumulative and non-cumulative excretion data were calculated from fecal chromium concentration. Viscosity was determined in digesta from birds and a section of the ileum removed for measurement of ileal morphological parameters. Feed type significantly ($P < 0.05$) improved body weight gain, feed intake and feed conversion ratio. Time of 50% (T50) and 1% (T1) chromium excretion were significantly ($P < 0.05$) decreased with enzyme addition. Non-cumulative excretion data yielded similar results. Enzyme supplementation significantly ($P < 0.05$) reduced retention time by 0.25h and increased ($P < 0.05$) feed passage rate, villus height and crypt depth in the chickens. Digesta viscosity was significantly ($P < 0.05$) reduced by enzyme addition. Results of the study showed that enzyme supplementation of diets improved digesta passage through the gut, improved ileal morphological characteristics and performance in broilers.

Keywords: feed passage rate, enzyme supplementation, digesta viscosity, broiler chickens

S1- 0075 Crude protein and energy requirements of the Japanese quail (*Coturnix coturnix Japonica*) during rearing period

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An experiment was conducted to evaluate the effects of diets containing different levels of metabolizable energy (3000, 3100 and 3200 kcal ME/kg) and crude protein (20, 22, 24 and 26% CP) on performance of Japanese quail. Two hundred and eighty eight 2-week quail chicks were assigned into 12 treatments of 24 chicks each. Three replicates were allocated to each dietary treatment randomly. For each level of energy, CP levels of 26, 24, 22 and 20 % were combined. Data on performance and nutrient digestibility were recorded and analyzed using a completely randomized design with a 4x3 factorial arrangement. Metabolizable energy (ME) significantly affected ($P < 0.05$) total and daily feed intake. Level of CP also had significant effects on the crude protein intake (CPI) and protein efficiency ratio (PER) of growing Japanese quails. Level of CP and ME had no significant effect on the body weight gain. The ME significantly affected ($P < 0.05$) the ether extract digestibility while CP that of ash digestibility. The results indicated that a diet of 26% CP and 3200kcal ME/kg is suitable for optimum performance of Japanese quail in terms of weight gain.

Keywords: Japanese quail, protein, energy, performance

S1- 0076 Comparative performance of broiler chickens offered diets with different protein and starch digestive dynamics as determined by Box-Behnken response surface design

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The rates and sites of protein and starch digestion are more indicative of growth performance than the extent of digestion in the small intestine of broiler chickens. Digestive dynamics of protein and starch may be influenced by diet composition and hours of feed access. The objective of this study was to use response surface methodology to evaluate the influence of digestive dynamics of protein and starch on growth performance of broiler chickens under different feeding regimen. Thirteen treatments were generated by Box-Behnken design with different inclusion levels of corn starch (0, 10, 20%), fishmeal (0, 8.75 and 17.5%) in sorghum-based diets and access to feed (6, 15 and 24 hours). A total of 390 Ross 308 chickens (mixed sex 1:1) were offered iso-caloric diets from 14-28 days post-hatch in bioassay cages. Feed intake was influenced only by hours of feed access with a quadratic relationship ($R^2 = 0.944$, $P < 0.0001$) where the maximal feed intake of 1720 g/bird was predicted by a feeding time of 22.01 hours. Weight gain was described by a second-order equation ($R^2 = 0.972$, $P < 0.0001$). $\text{Weight gain} = 259.4 - 5.055\text{starch} + 0.4743\text{fishmeal} + 86.52\text{feedingtime} + 0.3048\text{starch}^2 + 0.3589\text{fishmeal}^2 - 1.930\text{feedingtime}^2$ Hours of feed access had greater impact on weight gain than corn starch and fishmeal inclusions; the more pronounced influence was observed when feeding time was less than 15 hours. Fishmeal, which was the source of rapidly digestible protein, increased weight gain and corn starch had the least influence on weight gain. Similarly, feeding time had the greatest impact on FCR ($R^2 = 0.854$, $P < 0.0001$) where increasing feeding time and fishmeal inclusion improved FCR. Corn starch had little impact on FCR in high fishmeal diets and showed a quadratic impact on FCR in diets containing low fishmeal levels. This demonstrated that FCR may be influenced by the relative rates of protein and starch digestion and protein digestion is more influential than starch digestion.

Keywords: Box- Behnken design, digestive dynamics, protein, sorghum, starch

S1-0080 The effect peanut pod on digestive organs development and ileum bacteria population in broiler chickens

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It has proven that the use of moderate amounts of dietary insoluble fiber improves the development of the digestive organs of broilers. This experiment was designed to assess the hypothesis that feeding broiler with peanut pod as an insoluble fiber source will result in improved gut digestive capacity and growth performance. The experimental diets were consisted of a control diet and three diets containing 2.5, 5.0 or 7.5% peanut pod. The body weight gain, feed consumption and feed conversion ratio of broiler chickens were determined. The feed cost per kg live weight was calculated. Empty body weight and internal organs were weighed. The length of whole the intestinal tract was recorded. The ileum bacterial populations quantified using 'standard Koch's plate method'. The dietary peanut pod especially at 5% level decreased feed intake compared to the control group. In grower phase the best weight gain was recorded in the broiler chickens fed the diet contained 7.5% peanut pod. All the peanut pod containing diets decreased grower phase feed conversion ratio compared to the control group. In the finisher phase, the growth rate and feed conversion ratio did not affect by the experimental diets. The gizzard weight and gastrointestinal length was increased in the chickens fed 7.5% peanut pod. In the ileum, the birds fed 2.5 or 7.5% peanut pod had higher Lactobacillus and lower E. Coli populations compared to the control group, respectively. The optimistic effect of dietary peanut pod on the growth performance of broilers in this study was probably because of an improvement in digestive capacity and bacteria populations of gastrointestinal tract. On the other hand, the calculations in the current study showed that the feed cost per kg weight gain of broilers was reduced with peanut pod contained diets particularly at 5.0% level. This finding suggests that the economical income have to take into account while evaluating the dietary fiber effects.

Keywords: peanut pod, gut pH, ileum bacteria, broilers

S1- 0081 The effect of dietary rice husk on the intestinal morphology and bacteria population in broilers

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Insoluble fibre in broiler diet may improve the health of the digestive tract and bird welfare. This study was carried out to determine the effect of inclusion rate and particle size of rice husk in the diet of broilers on the intestine bacteria populations and morphology. The rice husk was divided in two parts with particle sizes less than 1 or 1-2 mm. The study carried out as a completely randomized design with 5 treatments, 4 replicates and 10 birds per replication. The experimental diets were consisted of a control husk free diet and four diets containing 0.75 or 1.5 percent rice husk with particle sizes of less than 1 mm or between 1-2 mm. Feed intake and weight gain were measured periodically and the feed conversion ratio was calculated. At 42 d of age, two birds of each repetition randomly selected and were slaughtered. Histological study was carried out on the small intestine. Ileal and cecal bacterial populations quantified using 'standard Koch's plate method'. The dietary type did not affect feed intake of the experimental groups. The best weight gain and feed conversion ratio were recorded in the broiler chickens feed the diet contained 1.5% rice hulls with particle size of less than 1 mm. In the duodenum and jejunum, the crypt depth to villi height ratio in the control group was significantly lower than other groups. In the ileum, all the birds fed rice husk except the group fed the diet contained 1.5% rice husk with particle size of 1-2 mm, had a higher Lactobacillus and lower E. Coli and coliforms populations than the control group. The results of the present study suggest that the 0.75% dietary inclusion and more coarse particles size of rice husk (1-2 mm) were more effective to promote broiler growth performance. The positive effect of dietary insoluble fiber on the growth performance of broilers was probably a result of changes in the bacteria populations of the gastrointestinal tract and not any improvement in small intestine absorptive capacity.

Keywords: rice husk, particle size, intestine morphology, gut bacteria population, broilers

S1-0082 Energy and protein requirements of Nigerian local hen reared under intensive system of management

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The Nigerian Local Fowls (NLF) are slow growers and are reared under extensive system of management. There is paucity of information on their dietary energy and protein requirements, and performance characteristics compared with improved breeds. A study was conducted to determine the energy and protein requirements of the Nigerian local hen reared under intensive system of management. In a 4×3 factorial arrangement using a completely randomized experimental design, 288 NLF at point of lay (24-28 weeks) were fed 12 diets at four Metabolizable Energy (ME) levels (2600, 2700, 2800 and 2900 Kcal ME/kg) and three protein levels (16, 18 and 20%) for 12 weeks. The performance, Apparent Nutrient Digestibility of Nutrients (AND) and internal and external qualities of egg laid by the hen were evaluated. Optimal egg weight (37.3g), albumen height (3.4mm), Haugh Unit-HU (70.6) and percentage Hen Day Production-HDP (59.5%) was obtained for hens on diet with 2700 Kcal ME/kg and 20% Crude protein (CP). The least Feed Conversion Ratio - FCR (2.7kg feed/kg egg) and cost of feed per egg produced (N3.9) were optimum for the same diet. The interaction between ME and protein had significant effect on FCR (3.0–6.2kg feed/tray of eggs), HU (64.3–73.1), Shell Thickness-ST (2.5×10⁻¹–3.2×10⁻¹mm) and AND of Dry Matter-DM (62.1–75.0%), Crude Fibre-CF (77.3–87.4%), and fat (87.3–93.1%; p < 0.05). Protein level had highly significant effect on HDP (32.9–62.3%), FCR (2.7–5.6) and AND of DM and CF (p < 0.01). The ME had significant effect on FI (58.2–69.8g/bird/day), FCR, ST, egg shape index (0.7–0.8), percentage albumen (44.4–53.5%), percentage yolk (36.0–44.7%), yolk index (1.6×10⁻¹–2.4×10⁻¹) and AND of DM, CP, CF and fat (p < 0.05). Indigenous hens required 2700 Kcal Metabolizable energy/kg and 20% crude protein for optimal performance and egg quality.

Keywords: Nigerian local hen, metabolizable energy, protein, performance, egg quality

S1-0083 Effects of dynamic segmentation dietary supply of nutrients on production and physicochemical properties of broilers

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This experiment was conducted to investigate the effects of dynamic segmentation dietary supply of nutrients on performance, carcass performance, immune organ index and intestinal structure of broilers. A total of 320 one-day-old broilers were randomly assigned into 4 treatments: A (every 14 days nutrition standard), B (every 7 days nutrition standard), C (every 3.5 days nutrition standard, but only 1 nutrition standard from 1 to 7 days) and D (every 3.5 days nutrition standard, and 2 nutrition standards from 1 to 7 days). Each treatment was represented by 8 replicates with 10 broilers per replicate. The experiment lasted for 42 days. The results showed that: 1) Performance: During 1 to 42 days, ADG of treatment A was significantly higher than B and C ($P < 0.05$), F/G of treatment A was significantly lower than C and D ($P < 0.05$). At 28 and 42 days of age, BW of treatment A was significantly higher than B and C ($P < 0.05$). 2) Carcass performance: Slaughter rate of treatment A was significantly higher than B ($P < 0.05$). Eviscerated percentage of treatment A was significantly higher than B and D ($P < 0.05$). 3) Immune organs indexes: There were no significant differences among 4 treatments in immune organs indexes ($P > 0.05$). 4) Intestinal structure: At 42 days of age, crypt depth of treatment A and B were significantly higher than C in duodenum ($P < 0.05$). Villous height/crypt depth of treatment D was significantly higher than A in duodenum ($P < 0.05$). In conclusion, the findings in this study reveal that performance and carcass performance of broilers are best when feeding with every 14 days nutrition standard. Every 3.5 days nutrition standard can promote small intestinal development of broilers. The immune function of broilers cannot be significantly affected by dynamic segmentation dietary supply of nutrients.

Keywords: broilers, dynamic segmentation nutrition, performance, immune organ index, intestinal development

S1-0084 Effects of in ovo feeding L-arginine on the hatchability and growth performance of broiler chickens

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Early development of the gastrointestinal tract is crucial for achieving maximal growth and development of broiler chicks. The embryo in the late hatching stage could naturally consume the amniotic fluids, in ovo feeding (IOF) nutrients into the embryonic amniotic fluid is an effective technology for regulating early nutrition supply and influencing its late growth. This study examined the effect of IOF different levels of arginine solutions into the embryonic amnion on the growth and development of chicks. On 17.5 d of incubation, 960 fertile Arbor Acres eggs (240 for each treatment, 55 g/egg) were injected with 600 μ L of 0% arginine (S group), 0.5% arginine (T1), 1% arginine (T2) or 2% arginine (T3), all dissolved in 0.75% saline, and 240 control eggs (C group) were not injected. After hatching, 80 male chicks from each treatment of eight replicates with 10 chicks for each replicate were placed in an experimental house. Results shows that T3 treatment decreased ($P < 0.05$) the hatchability rate than other treatments. T2 treatment increased ($P < 0.05$) the average feed intake and body weight gain of 1-7 d and 1-21 d, absolute weight and relative weight of yolk sac and duodenum of 1 d chicks, the absolute weight of liver, stomach, jejunum, spleen thymus gland and bursa of 21 d chicks compared with C group and S group. T2 treatment increased ($P < 0.05$) the relative weight of stomach, jejunum and ileum of 1 d chicks, the absolute weight of ileum of 21 d chicks compared with other treatments. T2 treatment also increased ($P < 0.05$) the relative gene expression of solute carrier family 7 (y+L system) member 6 (SLC7A6) and sodium/glucose cotransporter (SGLT-1) in duodenum, jejunum and ileum of 21 d chicks compared with C group and S group. In conclusion, IOF 1% arginine resulted in a significant improvement in the growth performance of male chicks with no affecting the hatchability rate.

Keywords: chicken, in ovo feeding, body weight, small intestine, amino acid transporter

S1- 0085 Selenium supplementation of broiler breeders influences fertility and progeny performances

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The present study compared two selenium (Se) forms: hydroxy-selenomethionine (HMSeBA) and Se-yeasts, fed to broiler breeders to determine their influence on egg production and fertility parameters and also the growth performances of the chicks' progeny. Total 200 females and 20 males at 54 weeks aged Ross 308 were used with 10 replicates in each group. Group 1 received a standard corn-soybean meal based diet supplemented with 0.2 mg Se/kg feed of Se-yeast containing 52% of its Se as selenomethionine (SeMet), while Group 2 received the same diet supplemented with 0.2 mg Se/kg feed of HMSeBA (> 99% HMSeBA) for 9 weeks. Eggs were collected daily and weekly assessed for quality and hen performances. At 58-59 and 61-62 weeks of age, 350 eggs (35 x 10 pens) per treatment were collected and incubated for hatching performances. Those chicks were then spread in 10 pens per treatment, and all fed a standard diet containing a mineral Se source, and growth performances were weekly recorded for 35 days. Results of egg production and fertility parameters did not indicate significant differences between the two treatments, but consistently over the different evaluation periods, performances of animals receiving HMSeBA were numerically higher than the Se-yeast group. Mainly, number of eggs produced, egg weight and feed conversion ratio were numerically higher throughout the study, as well as shell strength at the end of the study. Hatching performances recorded over two collection periods also indicated tendencies of improvement in the HMSeBA treatment group compared to Se-yeast group. Chick progeny performances indicated numerical improvement of body weight in the HMSeBA group over the two periods as well as improvement of the feed conversion ratio, compared to the Se-yeast group. The highly bioavailable Se form HMSeBA fed to broiler breeder indicated consistent tendencies of improvement of hen production performances, hatchability besides the chick progeny growth performances.

Keywords: selenium, broiler, breeder, progeny, fertility, performance

S1-0086 Performance response to dietary supplementation of protected butyric acid and a cocktail of protected organic acids and essential oils in laying hens

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Antibiotic use in egg-producing hens may lead to the transfer of drug resistant traits to pathogenic bacteria as a result of drug residues found in fully developed eggs. Presently, organic acids and essential oils are two of the most commonly used additives in lieu of antibiotics in laying hen production. A study was conducted to compare the effects of organic acids and essential oils, alone or in combination on laying hen performance during the late stage of production. A total of 252 80-week old Lohmann Classic were randomly assigned to receive 1 of 3 dietary treatments for 8 weeks: 1) basal diet with no antibiotic (T1) (n=84), 2) T1 + 1000 g/ton protected butyric acid (n=84), 3) T1 + 300 g/ton protected organic acids and essential oils (n=84). A completely randomized design consisting of 3 treatments, 21 replicates, and 4 hens in each replicate was used. Performance parameters including percentage hen-day egg production, feed consumption, egg weight, egg mass, and feed conversion were measured. Data were analyzed using the mixed model procedure with compound symmetry variance structure for repeated measurements. In terms of main effects, no significant differences were observed on measures of performance among dietary treatments. However, a trend towards improved egg production ($P<0.09$, 5.8%) and egg mass ($P<0.09$, 5.1%) were observed in T3 birds relative to T1 and T2 birds. Significant differences due to period were found for percentage hen-day production ($P<0.03$), feed consumption ($P<0.001$), egg weight ($P<0.001$), egg mass ($P<0.02$), and feed conversion ($P<0.001$). However, no significant differences were found for all measured parameters due to interaction of main effects. Overall, the dietary supplementation of protected butyric acid and a cocktail of protected organic acids and essential oils had similar effects on laying hen performance. Period differences may be due to the normal variation in laying performance caused by the changes in laying hens age.

Keywords: organic acids, essential oils, butyric acid, performance, laying hens

S1- 0087 Delayed post- hatch feed placement affects the performance and immuno- competence in meat type chickens

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Effect of delay in post-hatch (PH) feed deprivation (FD) for 6, 12, 24 and 36 hrs on the performance, immune organ development and immune-competence in meat type chickens (Coloured broilers) was studied. At 21 day, no difference was observed in body weight until 24 hr FD chicks while significantly decreased body weight was observed in 36 hr FD chicks. At 42 day, no significant difference was observed in control and FD chicks though it was lower in 36 hr FD chicks. Feed intake (FI) during 0-3 wks PH was lower in 36 hr FD chicks compare to the control and other FD chicks. The feed conversion ratio (FCR) of control or FD chicks didn't differ. At 36 hr, linear increase in the PCV and Hb as the FD period increased reported. Chicks FD for 24 and 36 hr showed significantly higher heterophil and lower lymphocytes compare to control chicks resulting in increasing trend of H: L ratio along with FD period. No difference was observed in the heart, bursa, spleen and thymus weight of FD and control chicks. In vivo humoral and cellular immune response was significantly better in chicks FD for 6, 12 and 24 hrs than control and 36 hr FD chicks. It may concluded that PH feed deprivation for first 24 hrs did not affect growth performance, immune organ development and immune response but feed withdrawal for 36 hr adversely affect the immune-competence in meat type chickens.

Keywords: feed deprivation, performance, immuno-competence, meat type chickens

S1-0088 In ovo supplementation improves the post- hatch performance, digestive organ development and intestinal morphology in early feed deprived meat type chickens

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The increased post-hatch holding period has the detrimental effects on chicks due to potential dehydration and energy depletion which may hampers the performance and genetic potential of the birds, which can be minimized by providing immediate feed to hatched chicks. Whether the knowledge of in ovo feeding (IOF) can be applied for ameliorating adverse effect of delayed feeding, the present study was carried out in which eggs were distributed in in ovo, sham control and un-injected control and chicks hatched from them are further divided in to immediate feed, 24 and 36 hr feed deprived (FD) chicks and response criteria were measured. Irrespective of period of fasting, at 24 and 36 hr PH numerically higher proventriculus, gizzard and intestinal weight, while significantly higher ($P=0.001$) duodenum VL and VW were found in IOF chicks than sham and un-injected control chicks. Irrespective of treatment duodenal VL and VW was higher in immediate fed chicks than the FD chicks. IOF and 24 or 36 hr FD chicks had higher digestive organ weight and duodenal VL and VW than respective un-injected control and immediately fed chicks. Irrespective of period of fasting, at 14 and 28d PH IOF chicks reported numerically higher BW and at 42d it was significantly higher than the un-injected control chicks. At 14d, chicks fed immediately or FD for 24 hr had significantly higher BW in comparison to those FD for 36hr. At 42d, IOF fed and 24 hr FD chicks had numerically higher BW than un-injected control immediate fed chicks. FCR was similar in all treatments and periods of fasting. It was concluded that irrespective of period of fasting in ovo supplemented chicks had better growth performance, digestive organ development and intestinal morphology than the un-injected control chicks. In ovo supplemented and 24 hr FD chicks had significantly better performance, while in ovo supplemented and 36 hr FD chicks had comparable result with that of un- injected control and immediately fed chicks.

Keywords: feed deprivation, post- hatch performance, intestinal morphology, in ovo supplementation, meat type chickens

S1-0089 Endocrine hydrogen sulfide enhanced protein anabolism of skeletal muscle in chicken

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For a long time hydrogen sulfide (H_2S) was considered a toxic compound, but recently H_2S (at low concentrations) has been found to play an important function in physiological processes. It regulates the cell cycle, apoptosis and the oxidative stress. However, it is not currently known that hydrogen sulfide regulates protein synthesis of skeletal muscle in chicken. The purpose of this study was to determine whether it accelerates protein anabolism in skeletal muscle. This study built two models to verify the effects of hydrogen sulfide on protein synthesis. Model 1: Different doses of DEX (dexamethasone, 0.1, 1, 10 μM) were utilized to hinder protein anabolism. When DEX (1 μM) successfully inhibited protein synthesis, L-cysteine (1 mM) was simultaneously supplemented to eliminate protein anabolic suppression induced by DEX. Model 2: In the presence of DEX, extra-supplement leucine with different doses (1, 10, 15 mM) was used to improve protein-synthesis rate. The leucine (15 mM) significantly eliminated the inhibition of protein anabolism by DEX. Next, this study used PAG (D, L - propargyl glycine, 1 mM) and leucine (15 mM) together to remove the effects of leucine on protein synthesis. DEX significantly inhibited myoblast protein-synthesis rate ($P < 0.05$) and abundance of CSE (cystathionine- γ -lyase) ($P < 0.05$). And L-cysteine successfully removed the effects induced by DEX. Conversely, when protein-synthesis rate was effectively elevated by leucine ($P < 0.05$), the expression of CSE mRNA significantly increased. But with PAG added to cultural media, the protein-synthesis rate and abundance of CSE were obviously decreased ($P < 0.05$). These findings at least partially confirm that hydrogen sulfide is involved in protein anabolic metabolism, and play an important role in the growth of skeletal muscle.

Keywords: hydrogen sulfide, protein synthesis, skeletal muscle

S1-0090 Apparent metabolizable and net energy values of corn and soybean meal for broiler breeding cocks

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The apparent metabolizable energy (AME) and net energy (NE) values of four corn varieties including two normal corn varieties (Zheng Dan 958 and Xian Yu 335), and each of waxy corn, and sweet corn, and two soybean meals including raw (RSBM) and dehulled soybean meal (DSBM) were determined by the substitution method in 2 experiments for broiler breeding cocks using the indirect calorimetry method. The test diets in Experiment 1 consisted of each corn sample by replacing 40% of the corn-soybean meal basal diet, and contained 25% RSBM or DSBM by replacing the corn basal diet in Experiment 2. Six open-circuit respiration chambers of approximately 0.43 cubic meter were used based on a design similar to that of van Milgen et al. (1997). Thirty (Experiment 1) or 18 (Experiment 2) 50-wk-old Arbor Acre (AA) broiler breeding cocks were used. After 7-d dietary adaptation period, six birds as replicates from each treatment were assigned to individual respiration chambers for energy measurement via gaseous exchange and total excreta collection for 10 days. The data were analyzed statistically via one-way ANOVA using the GLM procedure of SPSS 19.0. The heat production (HP), fasting heat production (FHP), and respiration quotient (RQ) were not significantly ($P > 0.05$) influenced by the various experimental diets in each experiment. In Experiment 1, The respective AME and NE values were 3785, 3775, 3738, and 3997 kcal/kg (DM basis), and 2982, 3006, 2959, and 3146 kcal/kg (DM basis) for Zheng Dan 958, Xian Yu 335, waxy corn, and sweet corn. The AME and NE values of sweet corn were significantly higher ($P < 0.05$) than those values of other three corn samples. In Experiment 2, the average AME and NE content were 2492 and 1581 kcal/kg (DM basis) for RSBM and 2580 and 1654 kcal/kg (DM basis) for DSBM, respectively. There was no difference ($P > 0.05$) in AME content between RSBM and DSBM. However, DSBM tended to have higher NE content than RSBM ($P = 0.06$).

Keywords: metabolizable energy, net energy, corn, soybean meal, broiler breeding cocks

S1-0091 The influence of eggshell as calcium source on egg and bone characteristics and serum profile of aged laying hens

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This experiment was conducted to determine the effects of eggshell as calcium source in aged laying hen diets on laying performance, egg and eggshell quality, serum Ca and P concentration and bone characteristics. A total of seventy five ISA Hisex Brown laying hens, were assigned to five treatment diets; including 100% limestone as control, 100% eggshell (particle size 2,0 mm and 0,5 mm) and 50% limestone with 50% eggshell both particle size respectively. Each treatment had five replicates with three hens each. After two weeks of acclimation, the birds were fed the experimental diets for five week. Initial and final body weights, feed consumption and feces production was recorded. Eggs production, weight and quality characteristics were measured. Serum Ca concentrations level were determined and finally, tibia contents of Ca and P concentrations were analyzed. Beside some differences among hens results of this experiment indicated that, there was no significant effect of dietary treatment on body weight and feed consumption. Replacing limestone in the experimental diets with fine and coarse ground, sterilized eggshell had no significant effect on egg production, egg weight and eggshell quality between treatments. Serum Ca concentration had the most significantly increase ($P=0,002$) in hens fed diet with 100 % coarse eggshell. The same treatment also caused the most significant increase ($P=0,022$) in the P content and decrease ($P=0,047$) of Ca content in tibia in relation to other treatments. It can be concluded that hatchery waste, as chicken egg shells, can be used as cheap and ecologically advisable Ca source in layer diets without an adverse effect on body weight, feed consumption, egg weight, egg production, egg and eggshell quality. Furthermore coarse grinding of eggshell the most effects on serum and bone mineralization, so more researches of this natural Ca source in aged laying hens are recommended.

Keywords: calcium source, eggshell, laying hens

S1- 0092 Comparison of the effects of probiotics and antibiotics on growth performance, growth of visceral organs, and serum biochemical parameters of Lohmann Brown chicks

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Better physiological health status at the early stage of growth and development could promote higher production and increase disease resistance during the laying period. Previously, few studies have reported the effect of probiotics on the growth performance and immune function of young layers, and this was the concern of our study. 540 1- day- old male Lohmann Brown chicks were randomly divided into three groups, with six replicates and 30 chicks in each replicate. The experiment was carried out over 70 days. Dietary treatments were: (1) basal diet alone (control group); (2) basal diet (probiotic group) containing 0.1% probiotic mix (*Bacillus subtilis* 1×10^5 cfu/kg of diet and *Pediococcus acidilactici* 1×10^5 cfu/kg of diet); and (3) basal diet containing 10 mg/kg zinc bacitracin (antibiotic group). Birds in the probiotic group had greater body weight (BW) than the control group during the overall experimental period ($P<0.05$). ADG (0-70d) was increased in the probiotic and antibiotic treatment compared with the control ($P<0.05$), whereas was not different between the probiotic and antibiotic group during the entire experimental period ($P>0.05$). The probiotic group had higher total protein (TP) level ($P<0.05$) than the control and antibiotic group on day 63. The length of duodenum, weights of spleen were increased in the probiotic and antibiotic groups as compared with the control group ($P<0.05$). The ileum was longer in the probiotic group compared with the control and antibiotic groups ($P<0.05$). The inclusion of probiotics in chicken feed could improve growth performance and visceral organ development, and enhance the health status at the early stage of growth and development of Lohmann Brown chicks. Compared with antibiotics, probiotics had a similar effect on promoting growth and might be a safer alternative to antibiotics as a growth promoter in chicks.

Keywords: probiotic, *Bacillus subtilis*, chick, growth performance, visceral organ

S1- 0094 Effects of raw materials grinding with disc mill on feed particle size and digestibility for broiler chicken

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In poultry farms, feed intake is essential to ensure an adequate supply of nutrients to meet the needs for growth or egg production. It is especially determined by feed particle size. A uniform feed particle size avoids a quicker intestinal passage of the feed in the digestive tract induced by too fine particles, and feed particle sorting caused by too large particles. In this context, disc mills, by crushing the raw materials rather than breaking them as with hammer mills, could improve feed homogeneity. To understand the effects of raw materials grinding with a disc mill, technological tests were carried out. Raw materials and premix of raw materials were ground on either a hammer mill or a disc mill with different settings of the interval between the discs. A trial was then performed on broilers from 21 to 24 days, in order to compare the feed metabolizable energy and protein digestibility depending on the milling method (hammer mill vs. disc mill) and byproducts (meals and DDGS) particle size. Technological tests show that disc mill reduces the proportion of fine particles in favor of coarser particle size fractions as compared to hammer mill. The discs' settings have a moderate effect on byproducts, but they modify the particle size profile of cereals and peas, without producing too much fine particles. Finally, the feed protein digestibility is significantly improved with disc mill compared to hammer mill (82.2% vs. 80.7%, $P < 0.001$). In conclusion, disc mill reduces extreme particles in favor of the intermediate particles and allows a greater flexibility to adjust feed particle size to the animal's needs, resulting in improvement of feed protein digestibility.

Keywords: raw materials grinding, feed particle size, digestibility, broiler chicken

S1-0095 Prediction of nutrition impact on broiler chickens performances by statistical modeling

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Nutritional programs have to be adapted to the evolution of broiler genetics potential. Grouping together trials results thanks to a modeling tool represents an undeniable asset to define the optimal strategy to reach production objectives. In order to achieve this nutritional modeling, fifteen zootechnical trials carried out in an experimental facility between 2005 and 2011 on the male breed Ross PM3 were compiled. The diets were standardized on the same matrix of formulation. The performances (consumption, growth and feed conversion ratio) were calculated over the same periods of time, separating three phases: starter, grower and finisher. Performances analysis was carried out with a mixed model with fixed effects and with or without random effect related to trial. Then, the models were validated on external data. The performances of the "grower" and "finisher" phases seem to be more easily predictable than those of the "starter" phase. Database analysis shows that digestible lysine level is an essential element and interacts with the level of metabolisable energy.

Keywords: statistical modeling, growth performance, feed consumption ratio, broiler chickens

S1-0096 Optimizing the starter period in poultry production by spraying the feed with an innovative nutritional solution

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Poultry sector is facing numerous important topics, not only economical expectations to ensure suitable earning to farmers but also social one, as poultry welfare improvement and antibiotic consumption reduction. Starter period represents a key phase for poultry. A poor starter period management can lead to poor future performance, high mortality rate and frequent antibiotics treatments. A nutritional concept was developed to help farmers to manage this starter period as well as possible. The sprayed solution aims at moistening the feed, coloring it in red to increase attractiveness and to stimulate chick feed intake and bringing highly digestible and energetic nutrients. Trials were carried out in experimental conditions on 700 Cobb broilers. Feed consumption increased during the first 3 days (+17%), which confirms the attractiveness for chicks. The chicks fed with the experimental diet were higher (+3.7%) than control chicks. Their residual yolk sac was reduced (-16%) compared to the control group, which means that there is a better vitellus use during the first 3 days. Fabricius bursa and heart weight were also higher (+7.6%, +11.6%) at 10 days of age. At 35 days of age, broiler weight and feed conversion rate were improved (+75 g, -0.02 points). Tested in several poultry commercial farms, the nutritional solution application led in performances improvement. Thanks to this sprayed solution, it seems to be easier to have healthy chicks and to reach better final performance.

Keywords: starter phase, hydration, feed consumption, poultry chick

S1- 0097 Gut microflora of broiler chickens depending on feed composition

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The present study was conducted to investigate the effects of antibiotic and probiotic on gut microflora of broiler chickens. 105 Cobb 500 day-old chickens were randomly divided into 3 groups of 35 birds each (1 control and two trial groups). Birds of the 1st control group received a standard ration according to Cobb 500 broiler management guide. Ration of the 2nd trial group was supplemented with 0.018% of Stafac-110 (antibiotic) and the 3rd trial group was supplemented with 0.1% of Cellobacterin-T (probiotic). At 1, 7, 14, 21 and 35 days of age, samples were collected from six birds per pen from each group by euthanasia. The contents of the small intestine and caecum were analyzed by terminal restriction fragment length polymorphism (T-RFLP) method with quantitative PCR analysis of 16S rDNA. It was showed that gut microflora has established on the first week of chicken's life. At the same time the total quantity of bacteria were 2.1×10^7 - 2.6×10^9 genomes/gram and depended from group. Changing of chicken's ration at 6-day of age caused increasing of bacterial communities in GIT such as Bifidobacteriaceae by 51.9%, Bacillaceae by 22.8%, Veilonellaceae by 42.8%. In addition, it also caused decreasing of Lactobacillaceae by 84.47%, Ruminococcaceae by 11.2%, Enterobacteriaceae by 98.8%. However, birds of the 2nd and 3rd trial groups had 1.2-2.7 times more populations of Ruminococcaceae and 3.2-8.3 times for Lactobacillaceae in GIT compared the 1st control group. Changing of chicken's ration at 15-day of age caused increasing of bacterial communities in GIT such as Ruminococcaceae by 52.6%, Lactobacillaceae by 70.0%, Bifidobacteriaceae by 76.6%, Bacillaceae by 51.0%, Veilonellaceae by 84.1% and Enterobacteriaceae by 62.6%. However, birds of the 2nd and 3rd trial groups had 46.6-82.8% lower populations of Enterobacteriaceae in GIT compared the 1st control group. It can be concluded that both antibiotic and probiotic in broilers has improved the GIT microflora.

Keywords: broiler chickens, gut microflora, T-RFLP, antibiotic, probiotic

S1-0098 Effects of in ovo feeding of creatine pyruvate on hatchability, growth performance and energy status in broiler chickens

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The effects of in ovo feeding (IOF) of creatine pyruvate (CrPyr) on hatchability, growth performance and energy status of Arbor Acres broilers were investigated. Five treatments were arranged as non-injected control, saline-injected group, and IOF treatments that contained 3, 6 and 12 mg CrPyr dissolved in saline per egg, respectively. Fertile eggs were injected with 0.6 mL of sterile solution into the amnion at 17.5 d of incubation (17.5 E). After hatching, a total of 80 male chicks were randomly assigned into 8 replicates of 10 chicks within each treatment. The results showed that no effect on hatchability was observed among groups, whereas the hatching weight of 12 mg/egg CrPyr treated group was significantly higher than that of control ($P < 0.05$). At 21 d post-hatch, the bodyweight of 6 and 12 mg/egg treated group was higher relative to the control ($P < 0.05$). The relative weight of pectoral muscle were greater in 6 and 12 mg/egg injected groups than that in the control at 19 E; hatch; and 3 and 21 d post-hatch ($P < 0.05$), respectively. Liver glycogen level was increased in IOF of 6 and 12 mg/egg CrPyr groups at 19 E, hatch and 3 d post-hatch ($P < 0.05$), respectively, and this effect maintained until 7 d post-hatch in 12 mg/egg group ($P < 0.05$). Neither pectoral muscle glycogen nor glucose contents were altered within treatments ($P > 0.05$). Irrespective of dosage, the concentrations of creatine, phosphocreatine and creatine kinase activity in embryos were enhanced by IOF treatments at 19 E when compared with the control ($P < 0.05$). Meanwhile, glucose-6-phosphatase activity of liver in 6 and 12 mg/egg CrPyr groups was higher than that of the control at 19 E ($P < 0.05$). In conclusion, the results indicated that IOF of CrPyr, especially at the level of 12 mg/egg, could improve hatching weight, body weight and pectoral muscle weight until 21 d post-hatch which might result from simultaneously enhanced liver glycogen, muscle creatine and phosphocreatine reserves in broilers. **Keywords:** broiler, in ovo feeding, creatine pyruvate, growth performance, energy status

S1-0100 Thigh meat texture profile analyses change of broiler chickens fed zeolite coated with silver nanoparticles during cold storage in the refrigerator

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Poultry meat quality attributes may be affected by several factors such as genotype, rearing conditions and feed additives could affect on muscle metabolism as well as on chemical composition. Nanotechnology is quite competent and these materials may be used for assurance of food safety in different food products. The aim of this study was to evaluation meat texture profile analyses change of broiler chickens fed zeolite coated with silver nanoparticles during cold storage in the refrigerator. A total number of 375 one-day-old broilers (Ross 308) from a commercial hatchery were randomly assigned to 5 experimental groups in a completely randomized design (CRD). Experimental diets were following: 1) Basal diet (Control), 2) Basal diet supplemented with 1% zeolite 3, 4 and 5) Basal diet supplemented with 1% zeolite coated with 25, 50 and 75ppm nanosilver respectively. On d 3 after storage in the refrigerator, there were no significant differences between treatments on hardness, adhesiveness, springiness, cohesiveness and gumminess ($P > 0.05$), while chewiness value was influenced by treatment diets ($P < 0.05$). The lowest value of chewiness was recorded by birds fed control diet. On d 7 after storage in the refrigerator, the springiness, cohesiveness, gumminess and chewiness value were affected by treatment diets ($P < 0.05$) and at the same time as hardness and adhesiveness were not influenced by dietary treatment ($P > 0.05$). In conclusion, The results of this study showed that the use of nanosilver coated in salt as feed additive had a beneficial effect on the broiler chicken thigh meat.

Keywords: broiler, zeolite, nanosilver, meat quality, texture profile analyse.

S1- 0101 Gut metagenomic analysis revealed crucial role of cecum in divergent feed efficiency of chicken

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Interaction between host genetic variations and gut microbiota would affect the gut nutritional metabolism and then influence feed efficiency. In this study, individual laying performance and feed intake of two hundred and fifty two brown-egg dwarf hens were recorded to evaluate feed efficiency. Hens with divergent feed efficiency, 14 birds per group, were selected to investigate their duodenal, cecal and fecal microbial composition by high throughput pyrosequencing the V4 region of 16S rRNA gene. The results showed that the microbiota in duodenum, cecum and feces were dominated by Firmicutes, Bacteroidetes and Firmicutes, respectively. And the Shannon index, which indicates the community diversity, of cecum was higher than duodenum and feces. In cecum, the abundance of a genus of Lactobacillaceae, Lactobacillus, Halomonas and Dechloromonas were greatly higher in high feed efficiency group, while a genus of Alcaligenaceae and Faecalibacterium were higher in low feed efficiency group significantly. These results indicated the crucial role of cecum in feed conversion efficiency in chickens, and also suggested the plausible functions of Lactobacillus related bacteria group in metabolism.

Keywords: feed efficiency, duodenum, cecum, feces, intestinal metagenomics, chicken

S1- 0102 Effects of Vitamin E supplementation on the performance, immunity and HSP70 gene expression in broiler chicken during hot-dry summer

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Climate resilience poultry production, especially management of heat stress is one of the major constraints for future development of poultry in tropical countries like India. Heat stress compromise immunity as well as production and leads to expression of heat shock proteins. To follow the suitable management system in low-cost open houses is very difficult therefore dietary approach seems to be more user-friendly to curb heat stress. Therefore to assess the efficacy of vitamin E for ameliorating heat stress, an experiment was conducted involving broiler chicks (n=120), reared in cages on a standard diet up to 14th day of age and thereafter up to 42nd day (14-42d) on test diets with or without vitamin E. The 14th day old chicks were randomly distributed into three dietary treatment groups viz., T1 (control diet), T2 (vitamin E @ 150mg/kg) and T3 (vitamin E @ 250mg/kg). Each dietary treatment comprised of four replicates with eight birds each. Further additional replicate per treatment was maintained to carryout blood metabolites and HSP70 gene expression. Experiment was carried out during hot-dry (April – May, 31.0 ± 0.70 to $37.0 \pm 1.40^\circ\text{C}$, RH: 58.0 ± 1.3 to $70.1 \pm 0.6\%$) summer. It was found that feed conversion ratio was improved significantly ($P < 0.001$) in all supplemented group during all phases. Cellular as well as humoral immunity improved ($P < 0.001$) due to vitamin E supplementation. The percentage of hemoglobin was ($P < 0.001$) improved, While H:L ratio and serum corticosterone decreased ($P < 0.001$) in vitamin E supplemented groups. Supplementation of vitamin E at the both levels caused ($P < 0.001$) down regulation of relative expression of HSP70 in jejunum tissues during 28th and 42nd day. Based on this study it was concluded that supplementation of 150mg/kg of vitamin E in broiler diets significantly improved their performance and welfare during heat stressed conditions.

Keywords: Vitamin E, broilers, heat stress, HSP70, production performance

S1-0106 The effects of fermentation and enzymatic pre-digestion of pea on nutrient digestibility in broilers

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Fermentation with probiotics and enzymatic pre-digestion may offer an interesting perspective to improve the nutritional quality of pea. This study examined the impacts of different inclusion levels of raw, fermented or enzymatic pre-digested peas on nutrient digestibility in broilers. For the fermentation, pea was mixed with water (1:1) containing 2.57×10^8 *Bacillus subtilis* (GalliPro[®]) spores/kg pea and then fermented for 48 h at 30°C. For the pre-digestion, pea was soaked in water (1:1) containing 3 enzymes, AlphaGal[™] (α -galactosidase), RONOZYME[®] ProAct (protease) and VP (pectinases), and incubated for 24 h at 30°C. Nine corn-wheat-soy diets were formulated by supplying 10, 20 and 30% of the crude protein with raw, fermented and pre-digested peas. The performance was recorded and the apparent ileal digestibility of Ca, P, K, protein, AAs and fat were measured at d 35. Data were subjected to ANOVA using the GLM procedure (Tukey test). Both types of processes reduced the raffinose equivalents, trypsin inhibitor and resistant starch. Increasing level of pea products reduced BWG and FI. Broilers fed pre-digested pea had the best FCR at d 35 ($P \leq 0.05$). Both processes had an identical effect on ileal digestibility of all nutrients except starch. The ileal digestibility of starch in raw pea was lower compared with both processed groups. The inclusion levels of products had no effect on digestibility of most nutrients. The digestibility of Thr, Lys and Met were higher in 30% groups compared with 10% groups, while chicken fed 10% products showed highest digestibility of starch ($P \leq 0.05$). In conclusion, both processes could relatively improve the nutritional quality of pea. Replacement of soybean by pea products at less than 20% inclusion level might have no negative impact on the nutrient digestibility and growth performance. These indicate the feasibility of both processes for nutritional quality improvement of pea as a partial replacement for soybean in broiler feed.

Keywords: fermentation, enzymatic pre-digestion, pea, broiler, anti-nutritional factors

S1-0107 Paradigm shift in feeding layer birds for omega 3 eggs production

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Poultry layer diet formulation based on fishmeal were incorporated with flaxseed oil (0 (control, 1.5%, 3% and 4.5%)). Individual omega-3 and omega-6 fatty acids were extracted from eggs from 39 weeks of ages of layer and analysed using Gas Chromatography technique. Egg quality was also determined as the level of flaxseed oil increased in the diet in addition to storage duration of eggs over 14 days. The results showed that by substituting ingredients such as maize, soyabean meal and fishmeal in layer diet with 3% flaxseed oil, there was about 0.2 mg/ml deposition of omega-3 and omega-6 fatty acids in chicken eggs. The level of deposition of omega 3 and omega 6 was 2.54 fold increase from the control with no flaxseed oil compared to 3% addition of the oil. For individual n-3 PUFA levels, the fold increases from control diet to the 3% oil inclusion were: 18:3 n-3 (α -linolenic acid, (ALA) = 1.55; 20:3 n-3 (Eicosatrienoic acid, (ETE) = 16.79; 20:5 n-3 (Eicosapentaenoic acid, (EPA) = 0.15; 22:3 n-3 (docosatrienoic acid) = 27.81; 22:5 n-3 (Docosapentaenoic acid, (DPA) = 1.6; and 22:6 n-3 (Docosahexaenoic acid, (DHA) = 3.22. The fold change in certain PUFAs were low because they were not different from the control. Haugh unit value which is a measure of protein quality in the albumen of eggs in the 3% addition of flaxseed oil was 81.3% diet compared to the rest of the treatment (0 (80.8%), 1.5 (80.0%) and 4.5 (80.8)). When eggs were stored over a period of 14 days in ambient temperature there was strong reduction in albumen quality ($y = -2.3444x + 97.15$, $R^2 = 0.96$) with increasing days of egg storage. This was possibly due to the increasing loss of moisture as egg storage duration increased in ambient temperature ($y = 8.6356x + 5.1478$, $R^2 = 0.56$).

Keywords: layer, feed, omega 3, omega 6, eggs

S1- 0108 Interaction between whole wheat inclusion and a conventional dose of microbial phytase on performance and nutrient utilization in broilers

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The intention of this study was to examine the influence of the main effects of whole wheat (WW) inclusion, phytase addition and their interaction on the performance and ileal nutrient digestibility in broilers fed pelleted diets. The experimental design was a 3 × 2 factorial arrangement of treatments evaluating three different feeding methods of wheat and two inclusion levels of phytase. A wheat-based diet was subjected to three different wheat inclusion methods: 622 g ground wheat/kg of diet and fed from d 1 to 21 (GW); 250 g/kg WW replaced ground wheat (w/w) pre-pelleting and fed from d 1 to 21 (PRP); and 250 g/kg WW replaced ground wheat (w/w) pre-pelleting for the first 10 d and then post-pelleting from d 11 to 21 (PRSP). These diets were then used to develop six dietary treatments using two inclusion levels (0 or 500 phytase units [FTU] per kg of feed) of phytase. A total of 288, one-d-old male broilers (Ross 308) were allocated to 36 cages (8 broilers per cage) and the cages were randomly assigned to the six dietary treatments. Birds fed PRP and PRSP diets gained the highest and lowest ($P < 0.05$) weights, respectively, with those fed GW diets being intermediate. Feeding PRSP diets reduced ($P < 0.05$) feed intake compared with GW and PRP diets. Birds fed PRP and PRSP diets showed similar feed per gain but lower ($P < 0.05$) than GW diets. Phytase addition increased ($P < 0.05$) weight gain and feed intake, and reduced ($P < 0.05$) feed per gain. Post-pelleting WW inclusion increased ($P < 0.05$) the apparent digestibility of protein and calcium, and phytase enhanced ($P < 0.05$) that of protein and fat. Phytase supplementation increased ($P < 0.05$) starch digestibility in PRP diets, but not in GW and PRSP diets. Overall, the current results showed that there is substantial opportunity to enhance nutrient digestibility by post-pelleting WW inclusion.

Keywords: broilers, nutrient utilization, phytase, whole wheat.

S1- 0109 Enzymological mechanism on sinapine-degrading for a strain isolated from intestinal tract of laying hen

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This article aims to the enzymological mechanisms of sinapine-degrading for a strain named YD-1 isolated from the intestinal tract of laying hens. YD-1 was vaccinated into the LB-Broth-Medium to prepare the extracellular products and the total protein of the extracellular products was determined by Coomassie Brilliant Blue. The activity of protease, lipase, lecithin enzyme, amylase, urease and gelatin enzyme in extracellular products were assayed qualitatively by the color and the area of the transparent circle after vaccinating the extracellular products into the Agar Plating of skim milk, Tween80, yolk, starch, urea and gelatin, respectively. Laccase activity was assayed qualitatively by the change of the color for bacterial liquid after vaccinating YD-1 into the bouillon sinapine culture-medium which contained ABTS(2,2'-azino-bis(3-ethylbenzthiazoline-6-Sulfonic acid)). Identified laccase gene of YD-1 by 16S rRNA and obtained the phylogenetic tree. Total protein in the extracellular products of YD-1 was 1.99 g/L. There appeared the colorless transparent, yellow transparent and yellowish-brown hydrolyzation circle in the agar plating hole of skim milk, starch and urea, respectively. Color changed to green from colorless for bacterial liquid of bouillon sinapine culture-medium after overnight cultured. Electrophoresis results of PCR products showed that YD-1 had the characteristic band of the laccase gene. Homologous analysis showed that the laccase gene of YD-1 shared 99% homology with the laccase gene of *Escherichia coli*. Accordingly, the extracellular products of YD-1 have the activity of protease, amylase and urease. YD-1 contains the laccase gene and can secrete laccase. The laccase of YD-1 belongs to *Escherichia coli* laccase. It is necessary to further study on the laccase gene expression of YD-1 and to study whether there has some else sinapine-degrading enzymes in extracellular or intracellular of YD-1.

Keywords: laying hen, sinapine, *Escherichia coli*, laccase

S1-0110 Efficacy of feed enzymes in maize–soya deoiled rice bran based diets on performance and immune–competence of Kadaknath chickens

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Effect of certain feed enzymes in maize-soy-deoiled rice bran based diets on performance, immunity and feed economics in Kadaknath chickens was studied. A feeding trial with Kadaknath chicks, consisting 4 dietary treatments: Basal diets- Control(C); C+Phytase+Protease; C+Phytase; C+Protease was conducted having 50 birds/treatments following CRD design. The supplemented rates of feed enzymes were phytase @ 100 mg/kg of feed and protease @250 mg/kg of feed. Response criteria include weekly body weight, feed intake and FCR. Humoral immunity (antibody titer against 1% sheep RBC) was studied in ten birds/ treatment on 28d. At first week, body weight didn't differ in control and other treatments but after second weeks it was significantly higher ($P < 0.01$) in phytase+protease treated group up to marketing age. Phytase and protease enzyme has comparable effect in body weight when fed individually. Weight gain was higher in phytase+protease treated group than other treatments. Feed intake in different treatments didn't differ, though it was higher in phytase and protease individually fed groups. No significant difference in FCR in different treatments though it was better in phytase+protease treated groups. Humoral immune response was significantly higher in phytase+protease treated group compare to control and other treatments. Feed cost/kg body weight was not differs due to dietary treatments. However, numerically less feed cost/kg body weight was observed in phytase+protease treated group than control. It was concluded that supplementation of phytase+protease in the diet of kadaknath was beneficial for improved growth performance, immunity and for reduction of feed cost.

Keywords: feed enzymes, Kadaknath, performance, immune-competence

S1-0111 Effects of dietary alpha–lipoic acid and acetyl–l–carnitine on growth performance, meat quality and anti–oxidative ability in broilers

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An experiment was conducted to evaluate the effects of dietary alpha-lipoic acid (LA) and acetyl-L-carnitine (ALC) on growth performance, meat quality and anti-oxidative ability in broilers. A total of 486 1-d-old male broilers were randomly assigned in a 3×3 factorial design using 3 LA and 3 ALC levels (6 replications, 9 birds/replication). The LA and ALC levels were 0, 50, and 100 mg/kg, respectively. Results showed that high level of LA and ALC led to lower ($P < 0.01$) average daily gain (ADG), average feed intake (AFI) and higher ($P < 0.01$) feed conversion rate (FCR). In addition, with increasing addition of LA or ALC, an decreased levels of L^* value and b^* value of breast muscle ($P < 0.05$), as well as decreased ($P < 0.01$) levels of pH 45 min and pH 24 h were observed in thigh muscle of broilers. Moreover, total antioxidant capacity, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities ($P < 0.05$) increased and malondialdehyde (MDA) ($P < 0.01$) decreased in serum and liver of birds with increased administration of LA or ALC. There was a synergistic effect of LA and ALC on the ADG, AFI, pH 24 h in thigh muscle, content of SOD and GSH-Px in serum. These results suggest that in combination supplementation of LA and ALC at low levels (50 or 100 mg/kg) can improve meat quality and anti-oxidative ability in broilers.

Keywords: alpha-lipoic acid, acetyl-l-carnitine, growth performance, meat quality, anti-oxidative ability

S1- 0112 Effect of frequency of administration of neem (*Azadirachta indica*) leaf aqueous extract on growth performance of broiler chicks

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One hundred and fifty one week old broiler chicks (Arbor acre) were weighed and randomly distributed to five dietary treatments A,B,C,D and E for growth rate determination. Each treatment was replicated three times on deep litter. The birds in treatment A were given vaccination and conventional drugs required for broiler chicks' production without neem leaf aqueous extract (NLAE) (control). Birds in treatment B were given vaccination and 10 ml of NLAE in 4 days interval without conventional drugs, while birds in treatments C,D and E were given 10 ml of NLAE at 4, 5 and 6 days interval, respectively with required vaccination and conventional drugs. The birds in all treatments were fed the same broiler starter diet ad-libitum. The experiment lasted for five weeks. Four hundred grams of neem leaves were used in the preparation of the NLAE and 10 ml of the NLAE was added to 250ml of water for birds I treatments B, C, D and E, each after which more water was served to all the birds in all treatments ad-libitum. Data generated were subjected to analysis of variance (ANOVA) and errors calculated as standard error of mean and significance accepted at 0.05 level of probability. The results showed that the birds in treatment A had the best final live weight (FLW) of 700.00g/bird, while birds in treatments B and E had the lowest FLW of 655.55 and 658.33g/bird respectively. The feed conversion ratio was best on birds placed on treatment B (3.37), unlike those placed on treatments A (3.54) and E (3.75). Cost of feed per kilogram live weight gain (CFPKLWG) was best on the birds in treatments B and D (₦349.92 - ₦350.53/kg). The administration of NLAE at 10ml/250ml of water at 4 days interval was not toxic to the birds as only 2 birds died in treatment B at starter phase (6,90% mortality). Broiler chicks can be reared with the administration of 10ml of NLAE in 250ml of water at 4 days interval without the use of conventional drugs

Keywords: frequency, neem leaf, growth, broiler

S1- 0113 Effect of frequency of administration of neem (*Azadirachta indica*) leaf heat treated aqueous extract on the carcass, organs weight and roundworm load of broiler chicken

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A 54 day feeding trial was conducted to determine the carcass characteristics and roundworm load of broiler chicken served neem (*Azadirachta indica*) leaf. One hundred and fifty, one-week old broiler chicks (Arbor acre) were weighed and randomly distributed into five dietary treatment groups A, B, C, D and E, and each treatment was replicated three times on deep litter. The birds in treatment A were given vaccination and conventional drugs without neem leaf aqueous extracts (NLAE). Birds in treatment B were given vaccination and 10 ml of NLAE at 4 days interval without conventional drugs, while birds in treatments C, D and E were given 10ml of NLAE at 4, 5 and 6 days intervals, respectively with the required vaccination and conventional drugs. The birds in all the treatment groups were fed the same broiler starter and finisher diets ad-libitum. Four hundred grams of neem leaves were used in the preparation of the NLAE and 10 ml of the NLAE was added to 250 ml of water for birds. Data generated were subjected to analysis of variance (ANOVA). The results revealed that the final live weight(FLW), plucked weight, dressed weight, breast weight, gizzard and large intestine weights were significantly ($p < 0.05$) different among the treatments, unlike plucked weight (% of live weight), shank and weight of small intestine ($P > 0.05$). The highest FLW was recorded on birds in treatment A and C (1500 g/bird), while the least was on the birds in treatment B (1350 g/bird). The breast weight (% eviscerated weight) and dressed weight (% of live weight) varied from 15.77-22.97 and 60.00-65.00. Hypertrophy was observed on the weights of the gizzard, lungs and for the birds placed on 10 ml NLAE at 4, 5, and 6 days interval relative to control and birds in treatment B. Presence of *Ascaris lumbricoides* (roundworms) was not observed in all the treatments.

Keywords: frequency, neem leaf, carcass, broiler

S1- 0114 Effect of different Levels threonine on growth performance, efficiency of carcass and morphology of the small intestine of broilers

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The purpose of this experiment was to evaluate the effect of different levels of threonine on growth performance, Efficiency of carcass and in broilers at the end of the period. Chicks (Ross 308, 1-d old) were randomly assigned to 4 treatment groups of 4 replicates each including 10 chicks per replicate in a completely randomized design arrangement. Chicks were offered four levels of threonine including: basal diet (without adding threonine), diets containing threonine as recommended by Ross 308 manual guide (level 0.05% threonine + basal diet , level 0.1% threonine + basal diet and level 0.15% threonine+ basal diet at Starter(1-10), Grower(11-24 days) and finisher(25-42 days) periods. Threonine effect on average daily gain, feed intake and feed conversion ratio were significant chickens ($P < 0.05$). The results showed the best performance in terms of average body weight gain, feed intake and feed conversion ratio of 1-42 days of treatment 0.1% of the threonine and the lowest yield of 1-42 days for the control (non-Thr), respectively. The morphology of the small intestine broilers was a significant difference between treatments ($P < 0.05$).

Keywords: threonine, growth performance, carcass efficacy, morphology of small intestine

S1-0115 Effect of different levels of raw, cooked and soaked peas on performance, efficiency of carcass and morphology of the small intestine of broilers

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This experiment was conducted to study the effect of different levels of peas with different processing methods was conducted on broiler chickens. The experiment was done in a completely randomized factorial design with 3 treatments and 3 levels and four replicates of the same energy and protein diets and a control treatment and with the amount of peas to zero, 7, 14 and 21%, and three peas raw, cooked or soaked replace corn and soybean meal. Processing treatments peas cooked for 30 minutes and soaked for 48 hours. Between daily gain and feed intake was significantly different between treatments and cooking and consumption levels peas significant effect on body weight of chickens fed with different treatments at the end of the experiment showed ($P < 0.05$). The feed intake of chicks under different treatments was not significant. Although the best FCR of treatments cooked peas and pea consumption level was 21%. In relation to the production of chickens tested were significant differences between treatments ($P < 0.01$) was observed. Differences in the amount and percentage of body treatments, including peas raw, cooked or soaked and interactive effects of treatments was insignificantly, however, significant differences between different levels of pea was observed ($P < 0.05$). The results of this experiment shows that can of peas as a good source of protein and energy supply used for poultry. The best method of treatment for the 21 percent of peas and cooked.

Keywords: raw, cooked and soaked peas, protein , broilers

S1-0116 Evaluation of different levels of NaCl on broiler chicken performance

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This experiment was conducted to study the effects of different levels of NaCl on growth performance on 360 male Ross 308 chickens. The chickens were divided into six experimental groups of four replicates, each of fifteen birds in Research Center of Azad University of Kashmar. Treatments included of NaCl levels (0, 0.1, 0.2, 0.3, 0.35 and 0.4 percent). Each treatment was kept in places with same conditions and all of chicken were kept free and had water and foods. After finishing 45 days of experiment, rates of growth increasing, consuming food and FCR were measured. Then, we made blood sampling from broiler chickens and rates of CBC, T3, T4, TSH and level of Cl and Na were measured using flame photometer. Data were analysed with SAS and Excel and for showing different of means, we used Donken test ($\alpha=5\%$). The largest increasing in weight of early periods was in third treatment, in middle periods was in first treatment and in final period was in second treatment. Food consuming in all of periods were influenced significantly and the largest food consuming was in third and fourth treatment. Fc was significantly in early period only so, in third treatment decreased 0.4 in comparison to control and in middle period in first treatment decreased 1 unit in comparison to control. Overall, with increasing NaCl, levels of serum Na also increased. ($P<0.05$)

Keywords: NaCl, different level, performance, broiler

S1- 0117 Effects of some optimized factors on different index of pellet quality and performance in broilers

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This experiment was done in order to investigate effects to changes in the production rate, grain particle size and steam conditioning temperature and adding moisture to the mesh on pellet quality and growth performance in broiler feed. In this experiment A 43 fractional factorial arrangement on grower and finisher feeds. Then, experiment design with Orthogonal array of L9 and data collection (PDI and Hardness in grower and finisher broiler diet) using Taguchi method was conducted. During the production process, sampling was done and then PDI and hardness evaluated in broiler feeds grower and finisher. Results of this experiment showed a significant effect of in the production rate, grain particle size and steam conditioning temperature and adding moisture on PDI and Hardness. In Conclusion the major finding of this experiment was recommend that for obtain performance and produce high quality pellet feed, adding moisture to the mesh, without the use of high temperature conditioning.

Keywords: broiler, PDI, hardness, conditioning, performance

S1-0118 The effects of synbiotic and phytogetic products on performance, gut morphology and blood characteristics of Japanese quails

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The aim of this study was to evaluate of synbiotic and phytogetic products on performance parameters, gut morphology and blood characteristics of Japanese quail. In a completely randomized design 180 day old Japanese quail were randomly allocated into 3 groups with 4 replicates of 20 chicks. Birds were assigned to three treatments 1) a control diet without supplementation 2) a diet with Biomin IMBO at a level of 1gr/kg 3) a diet with Biomin P.E.P at a level of 1gr/kg. Supplementation of synbiotic (Biomin IMBO) to the diets significantly improved the feed conversion ratio but did not cause any significant differences on body weight gain and feed intake. By using of probiotic total white blood cells increased significantly ($P < 0.05$), but the other blood parameters did not affected by levels of the probiotic and phytogetics products. Dietary synbiotics and phytogetics directly affect development of the gut. Significant differences ($P < 0.05$) among groups for intestinal parameters were found.

Keywords: synbiotic, phytogetic, quail, growth performance, gut morphology, blood characteristics.

S1- 0119 Comparison of protease benefits in corn or sorghum based broilers diets

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Ingredients prices and availability raise the need to optimize feed formulation by diversifying sources and getting the most out of them. Improving digestibility, protease use is of interest but its efficacy questioned in front of various raw materials. This study aimed to measure and compare benefits achievable with and without protease on either corn or sorghum based diets. In a 45 days study, 2,700 one day old mixed sex Cobb 500 broiler chickens were randomly allocated in a 2x3 factorial design with 2 different cereals: corn (C) or sorghum (S) and 3 diets formulations: positive control (PC) formulated on Cobb 500 nutritional level, negative control (NC) being PC reformulated with - 5% crude protein and digestible amino acids (AA), and a protease diet (PT) being NC + 125 ppm protease. This design resulted in 6 treatments (C-PC, C-NC, C-PT, S-PC, S-NC, and S-PT) with 9 replicates each of 50 birds. Body weight (BW), feed conversion (FC), mortality, carcass yield (CY) and skin pigmentation were analyzed weekly via Analysis of Variance (P value of 0.05) and separated using Fisher's LSD test. Throughout the study until d45, chickens fed corn were significantly heavier than the ones fed sorghum (2.572 vs 2.311 kg), had better FC (1.805 vs 1.929) and better CY especially for leg muscles. NC diets resulted in significant reduction of BW (2.401 vs 2.464 kg at d45) and CY for both cereals sources. Addition of protease on top of NC diets allowed recovering similar CY and BW than PC diets (2.460 vs 2.464 kg). Protease use lead to similar positive trend for both cereal sources with a significant recovery of FC and BW until d45 for sorghum based diets. Cereal source or protease addition had no effect on skin pigmentation or mortality. In conclusion, for similar nutritional levels, sorghum based diets lead to lighter animals than corn diets, use of protease on top of -5% CP and AA diets allowed recovery in performance with a more marked effect in sorghum based diets.

Keywords: protease, performance, broilers, corn, sorghum

S1- 0120 Protease benefits laying hens during heat stress

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Asia contributes to 58.8% of world egg production and regroups diversified climate countries. Heat stress (HS), observed when birds experience difficulties in achieving balance between body heat production and loss, occurs in hot climate as well as in temperate countries with sudden rises in temperature and/or humidity. Hens continually adjust feed intake (FI) according to temperature, and under HS conditions FI declines leading to poor egg production performance. Diet formulation is one tool to help hens coping with HS. The objective of this study was to investigate advantage of protease use on layer's performance under HS conditions. A total of 288 Shaver White layers, aged 45 weeks, housed in 96 cages of 3 layers, were homogeneously distributed in 2 groups supplemented or not with 125 ppm protease on top of a commercial diet. Production performance of each group, egg weight (EW), laying rate (LR), FI, egg mass (EM) and feed conversion (FC), were simultaneously recorded during a controlled HS challenge consisting of 2 replicated sequences of four phases: "Before", "During" HS, "After" (5 days each) and a "Break period" (10 d). HS challenge was proven effective and significantly ($p < 0.05$) decreased FI (81.5 vs 109.8 g/l/d). EW decreased within the HS phase (58.87 vs 59.36 g) and stayed low during the "After" phase. The LR stayed unchanged during HS phase and decreased only during the "After" phase and stayed low during the "Break period". Protease addition significantly improved FI (109.8 vs 108.2 g/l/d), LR (97.17 vs 95.53 %) and EM (57.68 vs 56.13) already during the "Before" phase. By comparison to the non-supplemented group, protease addition allowed significant regain in performance during the "Break period" phases following heat stress (LR of 95.24 vs 93.83 %). In conclusion, this trial showed that addition of protease, through better and quicker recovery of performance, could bring significant economic benefits under heat stress condition in laying hens.

Keywords: protease, performance, layers, heat stress

S1- 0121 Methionine- deficient diet alters performance, digestibility and gene expression of amino acids transporters in broiler chickens

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Methionine (Met) is the first limiting amino acid in a typical corn-soy poultry diet. Suboptimal levels of dietary Met can affect growth of poultry. We investigated performance, digestibility and molecular transporters of essential amino acids in chickens fed a Met-deficient diet. Cobb500 chicks were raised from hatch till 22 days in floor pens. At day 23, 10 birds were fed on a control diet (0.74% met+cys) and another 10 birds on a treatment diet (0.56% met+cys), and raised in individual cages. At day 41 all birds were euthanized and samples of Pectoralis (P.) major, kidney, ileum and hypothalamus were collected for gene expression analysis. The ileal contents were used for digestibility analysis. The GLM procedure was used for analysis. Chickens fed the Met deficiency diet had reduced growth, increased feed intake and worse FCR compared to controls. The apparent ileal digestibility (AID) of Met was similar between both groups. The AID of all other essential amino acids were significantly higher in the Met deficient group compared to the control group. The amino acid transporters b0,+AT and LAT4 expressions were upregulated in the ileum and kidney but TAT1 and LAT1 were downregulated only in kidney of the treatment group when compared to the controls. In the P. major, transporters SNAT1, SNAT2, SNAT7 and CAT1 were upregulated in the treatment group when compared to the controls. In the hypothalamus, SNAT1, SNAT7, LAT1, LAT4 and CAT1 were upregulated in the treatment group when compared to the control group. We can conclude that, dietary Met deficiency impairs growth and feed efficiency and increases the AID of all other essential amino acids except for methionine. Deficiency in dietary methionine also alters the amino acid transporters expressions in the ileum, kidney, P. major and hypothalamus. The expression differences could potentially reflect on the digestibility and availability of the essential amino acids in the methionine deficient group.

Keywords: methionine, digestibility, amino acids transporters, nutrigenomics

S1-0122 Manipulation of the dietary phytic acid, myo-inositol and exogenous phytase levels influenced growth performance, digesta pH and the gene expression profile in the jejunum of broilers

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The aim of the experiment was to study the extra-phosphoric effects of phytase in broilers receiving diets containing supplemental phytic acid (PHYT) and myo-inositol (MYO) using small intestinal gene expression and growth performance as response criteria. The experiment was a randomised complete block design in a 2 x 3 factorial arrangement, with treatments formulated based on a nutrient adequate control (C) diet, to which MYO or PHYT were added to increase the MYO and inositol phosphate ester content, respectively. To each of the 3 diets, phytase was added at 0 or 1500 FTU/kg. A total of 384 Ross 708 broilers were allocated to the 6 dietary treatments, with 8 replications per treatment, and feed and water provided ad libitum throughout the trial (hatch to day 21). On d21, following euthanasia, the pH of the duodenum, jejunum, ileum and caeca were taken *in situ*. Jejunal mucosal scrapings were used for quantification of expression of genes involved in nutrient transport. Body weight gain increased with phytase ($P < 0.05$) supplementation, but no other effects were reported on performance. Phytase increased ileal pH ($P < 0.001$). PHYT addition decreased caecal pH compared to birds fed the C and MYO diets, whereas phytase increased caecal pH (diet composition x enzyme $P < 0.01$). There was an interactive influence on the expression of NaPi-iiB ($P < 0.01$), increasing in the PHYT compared to C, only when phytase was absent. Expression of MUC2 increased with phytase ($P < 0.05$) and PepT1 decreased in diets supplemented with PHYT compared to the C ($P < 0.05$), but was not significantly different to diets supplemented with MYO. SLC34A2 expression in broilers receiving the C diet was similar to broilers fed the MYO and PHYT, but was greater in birds fed the PHYT than birds fed MYO ($P < 0.05$). Reducing phytate concentrations leads to enhanced gut integrity as well as increased Na and peptide transport which probably contribute to the benefits in gain seen with feeding phytase.

Keywords: super-dosing, phytase, myo-inositol, phytate

S1- 0123 Effect of methionine hydroxyl analogue chelate copper on gut health in broilers

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Copper (Cu) has been widely used at pharmacological levels (e.g., 125 ppm) as growth promoter in poultry. However, the mechanism by which high levels of Cu promote growth remains to be determined. It is commonly recognized that Cu exerts anti-microbial effect in the gut but there is not much direct and consistent data showing that Cu alters the population of bacterial species in birds. The objective of this study was to understand the mechanism by which methionine hydroxy-analogue chelate (MHAC) of Cu (MINTREX® Cu, Novus International, Inc.) improves gut health in broilers. Wheat-barley-SBM based diets were formulated to meet or exceed nutrient needs except for trace minerals. MHAC Cu was added to provide 0 ppm (NEG), 30 ppm (M30), or 75 ppm (M75) in final diets. Supplemental levels of Zn and Mn from MHAC (32 ppm), Fe (40 ppm), I (1.25 ppm), and Se (0.3 ppm) were similar among treatments. Each diet was fed to 9 replicate pens of male broilers. Data were analyzed by one-way ANOVA. Both M30 and M75 reduced ($P < 0.05$) FCR during grower phase (d 15-27). M75 reduced ($P < 0.05$) footpad lesion scores in birds of 33 and 40 d of age. Compared to NEG, M30 reduced ($P = 0.047$) villus width, and M75 reduced ($P = 0.034$) muscular width in the duodenum of birds of 21 d of age. Both M30 and M75 increased jejunal villus height ($P < 0.0001$), villus height/width ratio ($P = 0.017$), reduced crypt depth ($P = 0.018$), and crypt depth/villus height ratio ($P < 0.0001$) in birds of 40 d of age. These results indicate that MHAC Cu improved gut morphology and likely reduced the needs of energy and nutrients to maintain gut barrier function. Adding 30 ppm Cu from MHAC Cu increased ($P < 0.04$) the relative level of *Lactobacillus* in cecum. Taken together, supplementation of MHAC Cu improved gut health of broilers by maintaining better gut structural integrity and shifting the gastrointestinal microbiota to more beneficial balance, which leads to better growth performance and healthier footpads.

Keywords: chelated copper, gut health, microbiota, footpad lesion

S1- 0125 Effects of dietary supplementation with carnosine on growth performance, meat quality, antioxidant capacity and muscle fiber characteristics in broiler chickens

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This study was to investigate the effects of carnosine supplementation on growth performance, meat quality, antioxidant capacity and muscle fiber characteristics in broiler chickens. 256 one-day-old Arbor Acres broilers were randomly assigned to 4 groups with 8 replicates of 8 chickens each: group A, based diet; group B, C, D, based diets supplemented with 100 mg/kg, 200 mg/kg and 400 mg/kg carnosine respectively. The whole experiment lasted 42 days. The results showed that feed/gain in group D was decreased ($P<0.05$) in the starter period and whole period. The pH45min, drip loss and cohesiveness of thigh meat were improved ($P<0.05$) in group D, higher redness, lower cooking loss, shear force and hardness were observed in group C and D ($P<0.05$). In addition, the activity of T-SOD of thigh muscle in group D and GSH-Px in group C and D were increased ($P<0.05$), and MDA content was lower ($P<0.05$) at 21d; the activities of T-SOD, T-AOC and GSH-Px in group D were increased ($P<0.05$), and compared with group A, MDA and carbonyl contents were decreased ($P<0.05$) at 42d. Dietary carnosine decreased diameter and increased density of MyHC I, IIa and IIb ($P<0.05$) at 21d the similar trend was found in group C and D ($P<0.05$) at 42d. Furthermore, the ratios of MyHC IIa and IIb in group C and D were affected, and the ratio of MyHC I was increased at 42d ($P<0.05$). Carnosine increased mRNA expressions of CaN, NFATc1 and PGC1 α ($P<0.05$) just as that of MEF2C and Myf5 in group D at 21d ($P<0.05$), the expressions of CaM in group B, C, D and MyoD in group D were increased at 42d ($P<0.05$). These finding indicated that carnosine supplementation was beneficial to improve growth performance, meat quality and muscle antioxidant capacity of broilers. The improvement of meat quality might be realized by enhancing antioxidant capacity and changing fiber types, and the variation in fiber type might be related with the changes in the expressions of genes correlated with calcineurin-NFAT.

Keywords: carnosine, antioxidant capacity, meat quality, muscle fiber characteristics, calcineurin

S1-0126 Utilization of solid state fermented rape seed meal as a source of protein in commercial broiler diets

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Two experiments were conducted to study the possibility of utilizing processed (solid state fermented) rape seed meal (RSM) in broiler (Cobb 400) diets (1 to 42d of age). RSM is a good source of protein, but it contains glucosinolates, which depress bird performance. In Experiment 1 (Ex 1), maize - soybean meal control diet (CD) was prepared. Two diets with raw (RRSM) and processed RSM (PRSM) at 10% each were prepared. In Experiment 2 (Ex 2), a CD was prepared, either RRSM or RSM processed by two methods of solid state fermentation that differed in length of fermentation (RSMP1 and RSMP2) were included each at 3 levels (5, 10 and 15%). Each diet was offered to 10 replicates consisting of 5 birds housed in battery brooders. Body weight gain (BWG), feed intake (FI) and feed efficiency (FE) were recorded at weekly intervals. Slaughter variables (ready to cook yield, RTC; breast, liver and abdominal fat) were measured at 42d of age. The results of Ex 1 indicated that 10% RRSM depressed BWG, FI, FE, RTC, breast weight and increased liver weight. PRSM significantly reduced FI, maintained BWG and improved FE compared to those fed the CD. RTC was similar, while breast weight was higher in the PRSM group compared to those fed CD. Incorporating RRSM progressively depressed BWG, FI and FE with inclusion level in Ex 2. BWG was not affected by including RSMP1 and RSMP2 up to 10 and 5%, respectively but depressed at higher levels compared to the CD. The BWG in PRSM fed groups were higher than the respective level of RRSM fed groups. However, the RSMP1 and RSMP2 did not affect FE up to 15 and 10%, respectively. Incorporating RRSM progressively depressed the RTC and breast weights compared to the CD. These slaughter variables were not affected by feeding RSMP1 up to 15%. Based on the data, it is concluded that solid state fermentation improved the nutritional value of RSM, which could be incorporated up to 10% in broiler diet without affecting performance and slaughter variables.

Keywords: rape seed meal, broilers, body weight, feed efficiency

S1-0127 Effect of dietary supplementation of distillery dried grain soluble from rice on performance of commercial broilers and White Leghorn layers

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Poultry industry depends on soybean meal (SBM) as a source of dietary protein due to its uniform quality and ideal amino acid profile. Ever escalating cost and non availability of SBM force nutritionists to look for alternate ingredients like distillery dried grain with soluble from rice (RDDGS), which is fairly a good source of protein. Two experiments were conducted to study the possibility of utilizing RDDGS in broiler (1-35 d of age) and White Leghorn layer (24-47 weeks of age) diets. The RDDGS was procured from a local manufacturer containing 45.83% protein, 1.39% lysine, 1.18% methionine, 1.65% threonine, 1.90% isoleucine, 3.67% leucine, 3.26% valine and 3.35% agrinine. The RDDGS was incorporated at four levels (0, 5, 10 and 15%) in broiler experiment and 3 levels (0, 7.5 and 15%) in layer experiment. In broiler experiment 13 replicates containing 23 male chicks in each and in layer experiment 18 replicates containing 88 birds in each replicate were allotted to the test diets. In broiler experiment, incorporation of RDDGS significantly improved body weight gain (BWG) at 5% and feed efficiency (FE) at 5 and 10% compared to those fed the SBM control diet (CD) at 21 d of age. At 15% RDDGS, BWG was not affected, but the FE was depressed significantly compared to those fed the CD. BWG and FE at 35 d of age were not affected by incorporating RDDGS up to 10%, but both the parameters were depressed significantly at 15% RDDGS in broiler diet. In layers, egg production, feed intake (FI), FI per egg, FI per egg mass, periodwise egg mass and mortality were not affected by incorporating RDDGS at 7.5%. All these parameters were depressed in layers fed diet containing 15% RDDGS. Egg weight and body weight were not affected by the level of RDDGS in diet. Based on the results of both experiments, it could be concluded that RDDGS can be included up to 10% in broiler and 7.5% in WL layer diet without affecting their performance

Keywords: rice distillery dried grain soluble, broilers, WL layers, body weight, egg production

S1-0128 Effect of rate and extent of starch digestion on laying hen performance and digestive tract morphology

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The benefits of including slowly digested starch (SDS) in broiler diets have been demonstrated. However, little research has been done on its effects on laying hens performance. Thus, two sources of semi-purified starch differing in vitro digestibility (wheat, rapidly digested starch, RDS; pea, SDS), were chosen to formulate six test diets. The diets, identical except for differences in RDS/SDS ratio (100/0, 80/20, 60/40, 40/60, 20/80 and 0/100), were each fed to hens in 10 experimental units (2 cages, 6 Lohmann LSL hens per cage) for 20 weeks (26-46 wks of age). Treatments were assigned in a Complete Randomized Design and data were analyzed with regression analysis using SAS 9.4. Differences were considered significant when $P \leq 0.05$. With increasing inclusion of SDS in diets, feed intake increased linearly, while hen-day egg production, feed efficiency (g of feed/g of egg) and egg specific gravity responded in a quadratic fashion with highest, lowest and highest values achieved at 80, 40 and 60% SDS, respectively. Starch type did not affect egg weight or feather covering. Relative gizzard empty weight was affected in a quadratic fashion by SDS inclusion, with the highest value in 40% SDS; in contrast, jejunum and ileum weights responded in an opposite quadratic manner, with the lowest values in 40 and 60% SDS treatments. The same effect was observed for proportional jejunum and ileum lengths. The digesta content (% of body weight) of small intestine segments increased linearly with SDS, while crop content achieved a maximum weight (quadratic response) at 60% SDS. Crop and ileal pH decreased linearly with SDS inclusion suggesting increased fermentation in both locations. Relative weight of the pancreas increased and liver decreased linearly with proportion of SDS starch. In conclusion, starch digestion characteristics affected both production and digestive tract characteristics and warrants consideration when formulating laying hen diets.

Keywords: rapidly-digested starch, slowly-digested starch, semi-purified starch, egg production, fermentation

S1- 0129 Effects of BCAAs on the mTOR pathway in small intestine of broiler

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The experiment was conducted to investigate the effects of branched-chain amino acids (BCAAs) on the gene expression of mammalian target of rapamycin (mTOR) signaling pathway of broilers. Three hundred and eighty-four 1d broilers were randomly assigned into 4 treatments, 6 replicates in each treatment and 16 broilers in each replicate. Each treatment was offered one of the following diets with different BCAAs levels: 3.04% (3.04TM), 3.93% (3.93TM), 4.82% (4.82TM) and 5.71% (5.71TM), respectively. The ratio of Leu: Ile: Val is 1.8:1:1.2. The experiment lasted for 21 days and all birds were ad libitum. On days 7, 14 and 21, one bird in each replicate was slaughtered. Tissue samples were taken from duodenum, jejunum and ileum and frozen in -70°C until RNA isolated. RT-PCR was used to measure the genes expression levels (mTOR, p70 ribosomal protein S6 kinase [p70S6K], eukaryotic translation initiation factor 4E binding protein 1 [4E-BP1]). Data were analyzed with one-way ANOVA by SPSS 19.0 and $P < 0.05$ was considered as significant. The results indicated that, in duodenum, the expression of mTOR, S6K1 and 4E-BP1 in 3.93TM and 4.82TM were higher than 5.71TM and 3.04TM on 7, 14 and 21 days ($P < 0.05$), and mTOR increased with the BCAAs from 3.04 to 4.82% ($P < 0.05$). In jejunum, the mTOR, S6K1 and 4E-BP1 levels were decreased with the BCAAs increased from 4.82 to 5.71% ($P < 0.05$), S6K1 and 4E-BP1 were higher in 4.82TM compared with 3.04TM ($P < 0.05$) on 14 and 21 days, while no significant difference was found between 3.04TM and 5.71TM ($P > 0.05$). In ileum, all the treatments showed no significant difference in 21 days ($P > 0.05$). In conclusion, the expression of mTOR, S6K1 and 4E-BP1 increased as the level of dietary BCAAs was elevated from 3.04 to 4.82% in duodenum and jejunum. However, mTOR, S6K1 and 4E-BP1 expression will decrease when supplementation of BCAAs to diet with 5.71%.

Keywords: branched-chain amino acids, broiler, mTOR pathway, S6K1 and 4E-BP1

S1- 0130 Determination of nitrogen corrected apparent metabolizable energy of corn gluten meal for laying hen

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The experiment was conducted to determine nitrogen corrected apparent metabolizable energy (AMEn) of Chinese corn gluten meal (CGM) for laying hen. 10 CGMs were selected from 22 samples mainly consumed in Sichuan provinces in China with gross energy (GE) and neutral detergent fiber (NDF) as the indicators to eliminate samples of similar chemical composition. 176 laying hen (Lohmann Pink) of 60wk were housed in individual metabolism cages and randomly allotted to one of 11 diets with eight replicate per diet with a uniform laying rate of 95%. A corn-soybean meal basal diet was formulated according to NRC (1994). The test ingredient was incorporated into the basal diet with a proportion of 15% in sacrifice of the energy-generating ingredients (corn, soybean meal and rapeseed oil). Excreta was collected for 4 days after a 7-day adaptation period with total collection excreta method. In the selected CGM samples, The average content of GE was 5546.78 kcal/kg of DM. On DM basis, The average content of Crude protein (CP), ash, Crude fiber, NDF, acid detergent fiber (ADF), hemicellulose (HC), EE were 68.28%, 1.61%, 2.52%, 10.46%, 4.31%, 6.15%, 2.14% respectively. The AMEn of the 10 CGM ranged from 3182.74 to 4750.38(kal/kg, on DM basis) and with an average of 3823.45(kal/kg, on DM basis). The available simple prediction equation was: $AMEn = 2.27 * GE \text{ (kal/kg of DM)} - 9096.96$ ($r^2 = 0.62$, $p = 0.0066$, $SEM = 301.9$). Stepwise regression resulted in the following equation: $AMEn \text{ (kal/kg of DM)} = 2.0 * GE \text{ (kal/kg of DM)} + 127.1 * HC \text{ (on DM basis)} - 8295.97$ ($r^2 = 0.87$, $p = 0.0009$, $SEM = 192.1$). Removing HC from the model resulted in the following equation: $AMEn \text{ (kal/kg of DM)} = 3.9 * GE \text{ (kal/kg of DM)} - 89.5 * CP \text{ (on DM basis)} - 52.5 * ADF \text{ (on DM basis)} - 11600$ ($r^2 = 0.85$, $p = 0.0012$, $SEM = 197.6$). In conclusion, CGM was available as an alternative feed ingredient for laying hen. AMEn can be predicted well by its chemical composition and the best simple predictor was GE.

Keywords: nitrogen corrected apparent metabolizable energy, corn gluten meal, laying hen, regress equation

S1- 0131 Nitrogen corrected apparent metabolizable energy for corn Distillers Dried Grains with Soluble in laying hens

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The purpose of the current study was to investigate the nutritional composition of 10 corn DDGS differed in neutral detergent fiber (NDF), and gross energy (GE) selected from a total of 20 corn DDGS collected from the main feed plant and feed farm located in sichuan province. The experiment was conducted combining the difference method with the total collection method. 176 laying hen ((Lohmann Pink)) of 60wk, with an average laying rate of 95% were assigned to 11 treatments with 2 birds per cage and 8 replicate cages per diet in a randomized complete block design. A corn-soybean meal basal diet was formulated according to NRC (1994). Each test diet was formulated with a selected corn DDGS sample incorporated into the basal diet with a percentage of 20% by replacing the energy-yielding ingredients (corn, soybean meal and rapeseed oil). Excreta was collected for 4d after a 7d acclimation. The average content of GE, crude protein (CP), ether extract (EE), crude fiber(CF), NDF, acid detergent fiber (ADF), hemicellulose (HC) and ash were 5049.26kal/kg(4272.15-5344.93 kal/kg), 29.11% (26.33- 32.83%), 11.12% (2.18- 17.78%), 8.84%(7.16- 11.81%), 37.08 (30.37- 48.41), 13.53% (10.79- 21.49%), 23.55% (19.07- 27.80%), 6.07% (4.70- 8.74%) respectively on DM basis. The AMEn of the 10 corn DDGS is from 729.21 to 2535.37 (kal/kg of DM) with an average of 1920.29(kal/kg of DM). The available simple regress equation was $AMEn \text{ (kal/kg of DM)} = 1.45 \times GE \text{ (kal/kg of DM)} - 5413.69$ ($r^2=0.90$, $P<0.0001$ SEM=200.08). Stepwise regress analysis resulting the following equation with no intercept: $AMEn \text{ (kal/kg of DM)} = 0.82 \times GE \text{ (kal/kg of DM)} - 24.10 \times ADF \text{ (DM basis)} - 79.87 \times CF \text{ (DM basis)} - 201.94 \times \text{ash (DM basis)}$ ($r^2=1.00$; $P<0.0001$, SEM=83.49). In conclusion, corn DDGS is feasible used in laying hen diet as an alternative feed ingredient. The AMEn of corn DDGS could be accurately predicted from its chemical compositions and the best single predictor was GE.

Keywords: corn distillers dried grains with soluble, nitrogen corrected apparent metabolizable energy, laying, hen prediction equation

S1-0132 Effects of in ovo feeding of grapefruit seed extract on hatchability and ileal microbial population of broiler chickens

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Chicken embryos faced with high stress caused by excessive production of heat during the late days of egg incubation period. Therefore, the addition of antioxidant nutrients may improve the embryo status. Since it is difficult to control egg composition via breeder feed, in ovo feeding might offer a promising solution to provide developing embryos with the dietary additives. Grapefruit seeds is a great source of plant antioxidants such as hesperidin, a natural immune system stimulator. It is also contains vitamin C, sterols, tocopherols, citric acid, limonoids, and trace minerals. This project conducted to study the effects of in ovo feeding of grapefruit seed extract on hatchability and ileal microbial population of broiler chickens. Materials and Methods: A total of 120 fertile Ross 308 broiler breeder eggs, obtained from a commercial broiler breeder company Sari/Iran, were injected in the air sac on 18.5 d of incubation using an insulin syringe for the delivery of the grapefruit seed extract dissolved in 0.2 mL of sterile distilled water. Treatments were include: a) 1.5 mg GFSE; b) 3 mg GFSE c) a non-injected control; d) and a 0.2 mLs sterile distilled water injected control were included. Statistical analysis was conducted using the General Linear Models procedure of SAS software. Results and Conclusions: Results showed that in ovo feeding of grapefruit seed extract had not any effect on hatchability of broilers. However, ovo feeding of grapefruit seed extract decreased E.Coli population in ileum content of birds at 10 d, but, There was not difference in Lactobacilus population of broilers at 10 d. In ovo feeding results can be affected by many factors such as birds strain, breeder health and nutritional condition, injection room temperature, humidity, ventilation rate, site and time of injection, pH and osmolality of injected materials, etc. In conclusion, grapefruit seed improved beneficial microflora of birds.

Keywords: in ovo feeding, grapefruit, microbial population, broiler chicken

S1-0133 Effect of oregano essential oil on performance, carcass characteristics and blood biochemical parameters in broiler chicks

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An experiment was conducted to investigate the effect of marjoram essential oil on performance, Carcass Characteristics and blood biochemical parameters in broiler chicks (Arian). In a completely randomized design with 750 broiler chicks, 5 treatments [basal diet (control), basal diet + 100 mg per kg probiotic (protexin), basal diet + 150 mg per kg of antibiotic (Avilamycin) and two diets containing basal diet + 200 and 400 mg per kg diet marjoram essential oils] in 6 replicates of 25 chicks in each replication, were tested in 42 day period. Body weight, feed conversion ratio and survival rate were not affected by treatments. However, at 21 days of age, marjoram essential oil treatments compared to the other treatments showed significant differences. At the end of the period, the percentage of the various components of carcass such as the breast, thigh, liver and heart in female chicks, and spleen, liver, back and neck in male chicks were influenced by experimental treatments ($P < 0.05$). The blood parameters such as LDL, HDL, albumin, amylase, lipase, T3, T4, as well as calcium, phosphorus and other blood parameters were different significantly between the treatments. The results showed that marjoram essential oils at 200 milligrams per kilogram of diets in early, and in the final rearing period can be used in broiler diets according to most cases a positive effect on the performance, carcass and blood parameters, however, further investigation is needed.

Keywords: oregano essential oils, broilers, performances, immune responses

S1-0134 Effects of genistein on cholesterol metabolism in broilers fed a high-energy diet

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This study was conducted to investigate the effects of genistein on cholesterol metabolism in broilers fed a high-energy diet. 180 one-day-old health AA male broilers were used for a 42-day experiment. Chicks were randomly assigned into 3 treatments with 6 replicates of 10 males in each group. Chickens in the control group were fed soybean-corn based starter or finisher diets to meet nutrient requirements. Chickens in high energy treatment groups were fed with a high energy with 5% metabolism energy (provided by soybean oil) more than the NRC standard from day 11-42 in the presence or absence of genistein (50 mg/kg). The growth performance, carcass traits and biochemical markers related to cholesterol metabolism were determined. Our results showed that energy density and genistein had no effect on feed intakes and body weight gain during the experimental period. Compare with broilers in the control, high energy diet led to increased mortality rate from 1.67% to 5.56% during 22-42 days which was significantly decreased ($P < 0.05$) by genistein (5.56 vs 2.78, respectively). Biochemical analysis showed that broilers in the high energy density had increased total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), where the free fatty acid (FAs) were increased compared with that of controls. Genistein had no effect on high energy density diet induced alterations including TC, LDL-C, and FAs. In contrast, high energy induced increase in total glycerol was significantly diminished by genistein. The mRNA level of liver 3-hydroxy-3-methylglutaryl-CoA reductase was up-regulated by high energy diet, which was markedly reversed by genistein. Energy density and genistein had no effect on the expression of sterol regulatory element binding protein-2, low-density lipoprotein receptor and cholesterol 7 α -hydroxylase. More studies are needed to reveal the underlying mechanism that contributed to the beneficial effects of genistein on cholesterol metabolism in broilers.

Keywords: broilers, genistein, cholesterol metabolism, high energy

S1-0135 Effects of dietary rapeseed meal levels on growth performance and standardized ileal amino acid digestibility in meat ducks from 15 to 35 d of age

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This study was conducted to investigate the effects of dietary rapeseed meal (RSM) levels on growth performance and standardized ileal amino acid digestibility (SIAAD) in meat ducks from 15 to 35 d of age. Six hundred and eighty 15-d-old ducks were randomly allotted to 5 treatments with 8 replicates of 17 ducks per cage based on body weight. Five isonitrogenous and isoenergetic diets were formulated by replacing 0% (the control), 25%, 50%, 75% and 100% soybean meal protein with RSM protein on a digestible amino acid basis. The corresponding levels of RSM in diets were 0, 6.66, 13.32, 19.98, 26.64%, respectively. In increasing dietary RSM levels, body weight (BW) and average daily gain (ADG) linearly decrease ($P<0.001$), average daily feed intake (ADFI) presented linearly or quadratically change ($P<0.001$), while feed to gain ratio (F/G) linearly increased ($P=0.0078$). Ducks fed the diets with 50% or more replacing with RSM had significantly lower BW, ADG and ADFI, or higher F/G than ducks fed the control diet ($P<0.05$). At 35 d, the 4th primary wing feather significant linearly decreased ($P<0.001$), and the weight of thyroid gland linearly increased ($P<0.05$) by increasing dietary RSM supplementation levels. The apparent ileal digestibility (AID) and SID of essential amino acid and non-essential amino acid linearly decreased ($P<0.01$) as increasing of dietary RSM levels. Ducks fed 100% RSM diet had the lowest ($P<0.01$) AID and SID of amino acid than ducks fed the control diet. These results suggest that based on growth performance and SIAAD, meat duck's dietary RSM level should be lower than 13.32%.

Keywords: rapeseed meal, growth performance, standardized ileal amino acid digestibility, meat duck

S1-0136 Effect of advanced glycation end-products of wheat gluten on the GUT functions of broilers

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Background: The gastrointestinal tract (GIT or GUT) is a stress-sensitive target. Maillard reaction generates advanced glycation end-products (AGE), and they may potentially shift homeostasis towards oxidative stress and inflammatory responses. **Objective:** This experiment was conducted in order to investigate the effect of AGE of wheat gluten on the GUT structure and functions of broilers. **Methods:** 180 male Arbor Acre broilers were divided into three treatment groups including the control (CON), the original wheat gluten (OWG), and the AGE of wheat gluten. We determined growth performance, the apparent digestibility of protein in stool, ileum morphology, parameters of oxidative stress and inflammation immune response in serum, crop and ileum at 21 day and 35 day. Data was analyzed by the one-way ANOVA procedure of SPSS. **Results:** AGE treatment significantly reduced body weight (BW), average daily gain (ADG), and average daily feed intake (ADFI), and increased the feed conversion ratio (FCR) ($P<0.05$) of 21-day-old broilers. Data of ileum morphology showed no differences between treatments. However, the apparent digestibility of protein in those fed with AGE was significantly lower than in the control ($P<0.05$). AGE induced oxidative stress, with no inflammatory immune status change in ileum. For 35-day-old broilers, AGE treatment had a negative effect on the BW and FCR ($P<0.05$), and no effect on the ADG, ADFI, digestibility, ileum morphology, oxidative states, and the inflammatory immune status of broilers. No diarrhea was found in the animals in any of the treatments. **Conclusion:** Maillard reaction products of wheat gluten have negative effects on the GUT function of early stage broilers.

Keywords: wheat gluten, maillard reaction, GUT, oxidative stress, broiler

S1-0137 Effect of an antistress dietary supplement on the reproductive performance of layer breeders

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Various stresses are responsible for decrease productive and reproductive performance of breeders. Effects of an antistress composition, containing vitagene-activating nutrients (vitamins, minerals, carnitine, betaine, etc.; PerforMax/Magic Antistress Mix), supplied with water at 100g/100L at stressful periods of time, related to vaccinations, first egg laying, active egg production growth and peak of egg production on the Hy Line breeders from day 106 until day 448 were studied. There were 1938 layers and 176 cockerels in each group (control and experimental groups respectively). There was an increase by 2.5% of the egg peak production (96.9 vs 94.3%) achieving 266.9 eggs per hen housed and peak plateau lasted about 50 days longer than that in the control birds. Improved egg production was associated with increased weight of the oviduct in the experimental layers. FCR (feed per 10 eggs) was also improved by usage of the antistress composition and was better than the target for the line. Antistress composition usage was also associated with improved fertility at 16, 40, 48 and 56 weeks by 2.5; 2.7; 2.8 and 3.7% respectively. In the same experimental group the hatch of condition chickens improved at 26, 32, 40, 48 and 56 weeks by 3.6; 2.1; 3.4; 4.9 and 4.3% respectively. Therefore, additional usage of antistress composition via drinking water can help maintaining high productive and reproductive performance of poultry breeders.

Keywords: layers, stress, vitagenes

S1-0138 Effects of breed and age on the bone mineral compositions and association with bone strength of tibia in poultry

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The bone development is an important factors affecting on the chicken production. Selection for fast growing broilers in bone mineral compositions and association with in bone mechanical is unclear. The objective of this study was to investigate the effects of breed and age on the tibia bone mineral compositions and association with tibia bone mechanical by using selection for fast growing broilers and non-selection, with slow growing small and big size Chinese native chicken breeds. A total of 300 with 1-day-old chicks from Daweishan mini chicken, Wuding chicken and Avian broiler were fed to 20 weeks. The chickens were sacrificed at 4, 8, 12, 16 and 20 weeks to measure the body weight, tibia minerals compositions and mechanical properties. Daweishan Mini chicken had significantly higher tibia bone strength parameters, and with higher contents of calcium, magnesium, strontium and manganese and lower phosphorus, potassium and sodium contents while converse case were observed for the broilers in all time points. No breed or age effect on the other mineral contents were observed in three time points. The correlation analysis showed that there were positive correlations between bone strength and the contents of calcium, magnesium, strontium, zinc and copper, while the contents of phosphorus, sodium and potassium were negatively correlated with bone strength. In conclusion, selection for fast growing broilers has been increased depositing more phosphorus, sodium and potassium in tibia bone and association with lower bone strength. Non-selection Mini chicken breed had higher bone strength and association with higher tibia bone calcium, magnesium, strontium, zinc and copper contents. Our results implicated that supplement appropriate strontium in diet may improve the bone strength and health in broiler and layer.

Keywords: bone mineral compositions, bone strength, commercial broiler chicken, Daweishan mini chicken, Wuding chicken

S1– 0140 Supplying an antistress composition with water to decrease negative consequences of commercially-relevant stresses in rearing birds

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Effects of an antistress composition, containing vitagene-activating nutrients (vitamins, minerals, carnitine, betaine, etc.; PerforMax/Magic Antistress Mix) on the rearing birds were studied. The experiment was conducted with Hy Line rearing birds: 2000 females and 400 males in control and experimental groups. The control group was fed in accordance with Hy Line recommendations with the diet formulated to meet all requirements in major nutrients and energy. The experimental group had an additional antistress supply via drinking water at the level of 100 g per 100L of drinking water during stressful periods of time imposed by vaccinations, grading, and transfer to the breeding houses. The experiment lasted for 105 days. The usage of antistress composition positively affected testes development of 15-; 26- and 56-week old cockerels. The liver of experimental birds was characterised by a significant increase in vitamin A content at various ages. There was an increase in Ca content of the bones of female birds at 15 weeks indicating better Ca reserves for future egg production. Results of balance experiments indicated that females and males of the experimental group were characterised by improved usage of nitrogen, calcium and phosphorus from the diet. Indeed, usage of the antistress composition with water in period of chicken stress positively affected experimental birds.

Keywords: rearing birds, stress, vitagenes

S1-0142 Epigenetic effects of an antioxidant composition in layer breeder diet

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Epigenetic effects could have important role in the poultry production, but there is a lack of scientific studies in this area. The aim of the present study was to evaluate possible effects of supplying an antistress composition, containing vitagene-activating nutrients (carnitine, betaine, vitamins, etc.; Performax/Magic Antistress Mix) at 100g/100L drinking water to Hy Line breeders on their progeny chicks with specific emphasis to chick uniformity as an important determinant of rearing birds quality. There were 1938 layers and 176 cockerels in control and experimental groups and the experiment lasted from day 106 until day 448. The obtained data indicate that supplying the antistress composition to breeders was associated with a significant improvement of the uniformity (at day 28) in progeny chicks obtained from breeders of various ages: 26 weeks (81.3 vs 67.3%), 32 weeks (85.5 vs 76.8%), 40 weeks (83.2 vs 68.8%), 48 weeks (75.5 vs 68%) and 56 weeks (73.7 vs 62%). It is interesting to note that there were no difference in weight of day old chicks between groups independent on breeder's age. Therefore, it seems likely that changes in egg composition due to supplying to breeders with important nutrients, including methyl donors (betaine, methionine, vitamin B12, etc.), could have epigenetic effects of the progeny chicks.

Keywords: chicken epigenetics, vitagenes

S1- 0143 Effect of feeding stock of rice and alternative to corn on growth performance, intestinal microflora and nutrients utilization in broiler chicks

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The study was conducted to investigate the effect of dietary of stock of rices (whole rice and brown rice) an alternative to corn on performance, intestinal microflora and nutrients utilization in broiler chicks. A total of 600, 1-d-old male broiler chick(Ross × Ross) were randomly assigned into nine treatments with four replicate pens(30 bird/replicate pen) for five week. The treatment groups were basal diet with corn(T1), 15% whole rice group(T2), 30% whole rice group(T3), 15% brown rice group(T4), 30% brown rice group(T5). In chemical composition of whole rice, brown rice and corn, moisture content was higher brown rice than corn. Fat content was higher corn than whole rice and brown rice, and protein content was lower whole rice than corn and brown rice. Crude ash and crude fiber was higher whole rice than corn and brown rice, and energy content of corn, whole rice and brown rice was 2,944, 4,347 and 4,408 kcal/kg respectively, it was higher whole rice and brown rice than corn. No significant differences were found among the treatments for body weight, weight gain, feed intake and feed conversion rate of broiler chicks during the experimental period, but the body weight was higher in 15% and 30% brown rice groups (T5 and T6 groups). Feeding stock of rice did not effect of the Lactobacillus, Salmonella and E. coli concentration in the intestinal microflora of broiler chicks. The Dry matter (DM), crude fat(CF) and crude protein(CP) in nutrients utilization were shown the higher in chick fed 15% brown rice group than other group($P < 0.05$). These result indicated that 15% brown rice was effective the performance and nutrients utilization in broiler chicks.

Keywords: whole rice, brown rice, growth performance, nutrient utilization, intestinal microflora

S1-0144 Effect of the acidified drinking water on growth performance, gastrointestinal pH value and digestive enzyme activity in meat ducks from 15 to 35d of age

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This study investigated the effect of drinking water acidification by citric acid on growth performance, gastrointestinal pH value and digestive enzyme activity in meat ducks from 15 to 35d of age. One hundred and twenty healthy 14-day-old Cherry Valley meat ducks were randomly divided into five groups: Control, pH5.45, pH 4.45, pH3.50 and pH2.56 groups, based on body weight. The control group drank tap water with a pH of 7.80. To obtain the required pH, citric acid was added to tap water in groups pH5.45, pH 4.45, pH3.50 and pH2.56 by 0.21g/kg, 0.34g/kg, 0.68g/kg and 4.49g/kg, respectively. The trail lasted for 20 days, and 6 ducks from each group were slaughtered at the end for subsequent measurements. The results showed that the pH 2.56 group significantly increased ($P < 0.05$) feed intake(FI) and feed to water ratio (F/W), and decreased ($P < 0.05$)water intake (WI) comparing to the pH 7.8 group. Ducks in the pH 5.45 group and pH 4.45 group had the lower feed to gain ratio (F/G) ($P < 0.05$) than ducks in pH2.56 group. WI, F/W and the activity of lipase in jejunum showed significant quadratic response($P < 0.05$) as reducing of pH value in drinking water. The relative length of cecum presented a linear decreasing ($P < 0.05$) with the decreasing of pH value in drinking water. Citric acid supplementation in drinking water had no effect on gastrointestinal pH value ($P > 0.05$). These results suggested that the acidified drinking water (pH 5.45, pH 4.45 and pH 3.50) has a positive effect on the growth performance and digestive enzyme activity in meat ducks from 15 to 35d of age

Keywords: acidified drinking water, growth performance, digestive enzyme activity, meat duck

S1-0145 Effects of feed form on laying performance, egg quality characteristics, nutrient utilization and development of the small intestinal tract of two strains of Hy-line layer

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Our aims were to investigate the effects feed form on productive performances of two strains of Hy-line layer and further survey the mechanistic actions underlying this effect. A total of 512 25-week-age Hy-Line grey layers and Hy-line brown layers (216 hens per strain) were equally divided into four groups. All hens were fed the same composition and nutrient content with different feed forms (pellet or mash). Laying performance, egg quality characteristics, nutrient utilization and small intestinal morphology structure were measured at specific days. The laying rate, average egg weight and daily feed intake in Hy-line grey layers were higher when feeding the pellet diet compared with the mash diet. Higher laying rate and lower feed:egg ratio and average daily feed intake were observed for Hy-line brown layers fed the mash diet compared with the pellet diet. Average egg weight and feed conversion ratio were higher in Hy-line brown layers than in Hy-line grey layers. Feed form did not affect egg quality characteristics. However, strain, age and their interactions had a significant effect on eggshell thickness, eggshell strength and yolk color. Age and interactions between age and strain had also significant effect on haugh unit and eggshell percentage. The results of metabolic assay displayed significant interactions between feed form and strain in the mean apparent metabolic rate of crude protein. Higher apparent metabolic rate of calcium was detected for the birds fed the mash diet. Examination of the small intestinal morphology indicated significant interactions between feed form and strain in the villus height of duodenum and ileum and villus height/crypt depth (V/C) value of duodenum, respectively. The V/C value of ileum was higher for the hens fed the pellet diet than for those given the mash diet. No other differences were found among groups. In conclusion, our findings suggest that feed form may be a contributing factor for layer productive performance.

Keywords: feed form, laying performance, egg quality, nutritional metabolism, intestinal morphology structure

S1-0146 Effects of pH value in drinking water during 1 to 14 days on growth performance, gastrointestinal pH and digestive enzyme activity of meat ducks

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This study was conducted to investigate the effects of pH value in drinking water during 1 to 14 d on growth performance, gastrointestinal pH and digestive enzyme activity of meat ducks aged from 1 to 35 days. 120 one-d-old cherry valley ducks were randomly divided into 5 treatment groups: Control, pH5.45, pH 4.45, pH 3.50 and pH 2.56 groups. The control group drank tap water with a pH of 7.80. To obtain the required pH, citric acid was added to tap water in groups pH5.45, pH4.45, pH3.50 and pH2.56 by 0.21g/kg, 0.34g/kg, 0.68g/kg and 4.49g/kg, respectively. The experiment included 2 phases: the starter phase (1 to 14d) where the tap water contained different concentrations of citric acid and the growth phase (15 to 35 d) where birds were drank tap water to examine the compensatory growth of ducks after citric acid withdrawal. Body weight, average daily gain (ADG) and cumulated feed intake (FI) of ducks in the starter phase linear decreased ($P < 0.05$) as decreasing of pH value in drinking water, but ADG in growth phase increased. Citric acid supplementation in drinking water showed a linear ($P < 0.001$) or quadratic ($P < 0.001$) effect on the amount of drinking water (ADW) and feed to water ratio. Ducks drank the water with pH 2.56 had the poorest ($P < 0.05$) performance due to having the lowest ADW compared to the other four treatments. Citric acid supplementation in drinking water showed a linear ($P < 0.05$) increasing on jejunum and ileum pH value, while had no significant ($P > 0.05$) effect on the activities of amylase, trypsin, chymotrypsin and lipase in jejunum of ducks at 14d of age. These results suggested pH value in drinking water for ducks from 1 to 14d presented a negative effect on growth performance and gastrointestinal physiology, whereas it could improve the growth performance of duck from 15 to 35d.

Keywords: pH value in drinking water, growth performance, gastrointestinal pH, digestible enzyme activity, meat duck

S1- 0147 Effects of supplementing prebiotics on performance and carcass parameters in commercial broiler chickens fed diet with two different levels of energy

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Prebiotic confers the health benefit upon the host by enhancing nutrient absorption and intrinsic strength of the immune system. The present experiment was carried out to determine the effect of prebiotic supplementation on performance and stress parameters in commercial broiler chicken. A total of 240 day-old chicks (Vencobb) distributed randomly into 40 battery brooder pens (4 treatments, 10 replicates with 6 chicks in each). A maize-soybean meal based control diet was formulated and fed with or without supplementing prebiotic (1 L/1000 kg feed; Agroman Cytozyme Pvt. Ltd. Mumbai) and another diet with 2.5% lower ME was formulated and fed with or without prebiotic (1 L/1000 kg feed). Each diet was allotted randomly to all the replicates and fed ad libitum from 1 to 42 days of age. The weekly body weight gain and feed intake was recorded throughout the experiment. Approximately 4 ml of blood was drawn from the brachial vein of chickens selected from each replicate on 35th day of age for estimation of antioxidant responses. At the end of the experiment, 8 birds from each treatment were sacrificed to record the various slaughter parameters. The body weight gain (BWG) and feed conversion ratio (FCR) improved ($P<0.05$) in groups fed diet supplemented prebiotics compared to those groups fed diets without prebiotic during the 3rd and 6th weeks of the experiment. However, supplementing the prebiotic among the various dietary groups did not affect the slaughter parameters and stress parameters. Therefore, it is concluded that the supplementation of prebiotic resulted in improved BWG and FCR in commercial chicken. However, supplementing the prebiotics did not affect the slaughter and stress parameters among various dietary groups in the present experiment.

Keywords: broiler chicken, performance, prebiotics, stress parameters

S1- 0148 Aflatoxin deactivating effect of Turmeric (*Curcuma longa*) and Garlic (*Allium sativum*) in broiler

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Aflatoxin (AF) as a secondary metabolite of *Aspergillus* species, are carcinogenic and mutagenic for chickens. The major source of exposure to AF is via the ingestion of contaminated feed. Chickens are highly sensitive to the adverse effect of AF and it is causing economic losses in poultry industry. The objectives of this study was to find out the deactivating effect of turmeric powder (TP) and garlic powder (GP) in broiler during AF challenging and also to estimate the AF residues in liver and meat after slaughtering. Randomly selected, 14 days old cobb-500 broiler chicks (N=84) were equally divided into four dietary groups. Four isonitric and isocaloric experimental diets were formulated, where in diet-A: 1.5 mg of AF; in Diet-B 1.5 mg of AF and 1g of TP; in Diet-C 1.5 mg of AF and 1 g of GP contained per kg of feed and control diet having no AF, TP and GP. Each dietary treatment had 3 replications (n=7) and respective feed was offered to the broilers according to the strain standard for the duration of experiment (35 days). All experimental broilers were reared following same management practices. At 36 days of age all experimental broiler were slaughtered for examining the necroscopic changes in internal organs. Liver and muscle tissues were also collected to estimate AF residues. Weekly growth rate and FCR were studied during the experimental period. The results showed that there were significantly different ($P<0.05$) in average weekly gain and FCR among the dietary treatment. Relative liver and kidney weight showed significantly ($P<0.05$) highest in diet-A than the diet-B, diet-C and the control diet but relative spleen weight was not significantly difference ($P>0.05$) among the diets. The residues of AF found in muscle and liver was also significantly ($P<0.05$) higher in diet-A than the other groups, respectively. Practical application of this research is supplementation of TP in diet of broiler to prevent or reduce the effects of feeding AF contaminated diets.

Keywords: broiler diet, aflatoxin, deactivating, turmeric, garlic, residual effect

S1-0149 Effects of dietary glucosinolates concentration on laying performance, egg quality and nutrient utilization in laying hens

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The objective of this trial was to study the effects of glucosinolates from rapeseed meal (RSM) on laying performance, egg quality, and dietary nutrient utilization in laying hens. A total of 900 30-week-old Lohman pink-shelled laying hens were randomly assigned to 6 dietary treatments with 10 pens per treatment, 15 hens/pen with 3 hens/cage. The diets were formulated on a digestible amino acid basis, isoenergetic and isonitrogenous with different dietary RSM level: a corn-soybean basal diet: 0, 5.88, 11.76, 17.64, 23.52 and 29.40%. The glucosinolates in RSM was 15.62 $\mu\text{mol/g}$. The trial lasted for 16 weeks including 12 weeks accumulation phase and 4 weeks withdrawal phase by feeding the basal diet. During the accumulation phase, the egg production, average egg weight and ADFI were decreased with the dietary RSM level increase in a linear manner ($P<0.01$), but the feed to egg ratio was increased linearly ($P<0.01$). There was no difference in mortality among the treatments ($P>0.05$). The utilization of dietary dry matter, nitrogen and AME was decreased with the RSM level increased in a linear way ($P<0.001$), and the apparent amino acid availability also was decreased linearly ($P<0.001$), except that of Ile and Gly ($P>0.05$). Dietary RSM didn't affect the thickness of eggshell ($P>0.05$) except that the eggshell strength was the highest at 11.76% RSM ($P<0.01$) at 12 week. The egg albumen height and haugh unit was decreased in a linearly manner ($P<0.001$), and the egg yolk color showed a linear increasing ($P<0.01$) during 1 to 12 week. After a 4-week withdraw of RSM, there was no difference in laying performance and egg quality when compared with the control group ($P>0.05$). The suitable dietary RSM level should be lower than 11.76%, and a 4-week withdrawal of RSM should be considered.

Keywords: glucosinolates, laying hens, growth performance, egg quality, nutrient utilization

S1-0150 Effects of storage time on nutrient and utilization of newly harvested maize for broiler

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This study was to investigate the utilization of starch in newly harvested maize for broiler. The newly harvested dent, flint and mixed type maizes and a 1-year-stored maize were stored at ambient temperature. When batches of each cultivar were stored for 0,2,4,6,8 weeks, various characterizations were conducted to determine the variation of maize aging. Samples of maize were used as formulated starter diet of broiler. 96 7-day-old Ross 308 broilers were randomly divided into 4 treatments. At the age of 15 to 18 days, 3 broilers in every replicate were fed by metabolic diet. AMEn, and the ileal digesta was collected on day 18 to determine starch digestibility by the method of indicator. The microflora of cecum digesta was analyzed by 16s rDNA technique. Venous blood samples were obtained at the age of 17d to determine the blood glucose and insulin. The results showed that: the content of resistant starch in newly harvested maize dropped to the level which is the same as the control one after 2 weeks; the content of Water-soluble pentosan and vitro viscosity decreased significantly after 4 weeks. During the early storage time, newly harvested maize affected AME and AMEn for broiler significantly ($P<0.05$), after 2 weeks, the AME and AMEn of the flint and mixed maize reached peak value. The ratio of AME and AMEn between newly harvested maize and the control maize increased along with the process of storage; Newly harvested maize improved the digestibility of total starch compared with control maize at the beginning of storage ($P<0.05$). Newly harvested maize increased contents of plasma glucose after the maize stored for 4 weeks ($P<0.05$); The ratio of beneficial microorganism in cecum increased along with the process of storage. The result suggested that maize storage could help to improve the process of glycometabolism, and improve the AME of maize for broiler by changing the content of resistant starch.

Keywords: newly harvested maize, starch, storage, broiler, microflora

S1-0151 Relation between selenomethionine content in dietary selenium sources and selenium deposition in broiler muscle tissue

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The aim of this study was to investigate the relation between the selenomethionine (SeMet) content in two different selenized yeast products and L-selenomethionine on selenium deposition in broiler muscle tissue. Two different commercially available selenized yeast products were analyzed for SeMet content with HPLC-ICP-MS, after twofold enzymatic extraction (protease/lipase at pH 7.5) in a water bath (37° C). Selenium in the form of SeMet was 26% in SeYeast product A and 69% in SeYeast B. Male broilers were fed one of 4 treatment starter diets. All treatments had 4 pens with 5 animals per pen. Treatment 1 was supplemented with 0.2 mg/kg total Se from sodium selenite. Treatment 2 and 3 were supplemented with 0.2 mg/kg total Se from SeYeast products A or B, which resulted in a supplemented Se in the form of SeMet of 0.052mg/kg and 0.138 mg/kg for SeYeast A and B, respectively. Treatment 4 was supplemented with L-selenomethionine (Excential Selenium4000) at a dosing of 0.2 mg/kg total selenium, which equals 0.2 mg/kg Se in the form of SeMet. Representative samples of the left breast of 3 broilers per pen were taken on d14 and analyzed for Se content by ICP-MS. Results show Se content in broiler muscle for treatment 1 (sodium selenite) of 133 µg/kg Se. SeYeast A and SeYeast B showed 161 and 267 µg/kg Se, respectively. Treatment 4 (L- selenomethionine) showed the highest Se content in muscle with 337 µg/kg Se. The data show that the Se deposition in muscle is linearly correlated with the added Se as selenomethionine. Linear regression of the data shows a fit with a R² of 0.7186. This study shows that selenium deposition in broiler muscle tissue is related to the added selenomethionine in the diet (P < 0.0001). SeYeast with high SeMet content results in higher Se deposition compared to SeYeast with low SeMet content (P < 0.0001). L- Selenomethionine supplementation results in the highest Se deposition.

Keywords: selenium, broiler, selenomethionine

S1-0152 Comparison of slow drying and rapid drying on the physicochemical characteristics of corn and its nutritional value for broiler chicks

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The slow drying of grains such as corn maintains moisture at higher levels over a longer period, allowing endogenous enzymes to remain active. This can affect the physicochemical structure of the grain. In the present study newly harvested corn (JiFeng I) was dried to 14% moisture content at two speeds: half was dried rapidly over 3 days and the other half dried slowly over 21 days. Seven- day- old broiler chicks were assigned to two groups, one of which received the fast-dried corn and the other of which received the slow-dried corn. Slow-dried corn had higher values for starch, acid detergent fiber, neutral detergent fiber, and gross energy, but lower in vitro viscosity, water-soluble pentosan levels, and total pentosan levels. Drying time had no significant effect on the growth performance or digestive physiology of chicks, although slow-dried corn had higher apparent metabolizable energy corrected for nitrogen and higher apparent nitrogen retention (P < 0.05). Slow-dried corn significantly decreased crypt depth in the duodenum (P < 0.05) and growth hormone concentration in serum (P < 0.05). In conclusion slow drying contributed to increased nutritive value of corn, decreased anti-nutritional factors in corn, improved the energy utilization and apparent nitrogen retention for in broiler chicks.

Keywords: corn, drying time, nutritional value, anti-nutritional factors, broiler chicks

S1-0153 Effect of different stored-moisture maizes on chemical content and nutritional value for broiler chickens

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In the north of China, most maizes were stored with high moisture content through the winter. While high moisture content storage could affect the properties of Maize. This study was conducted to investigate different moisture-stored Maize on chemical content and nutritional value for broiler chickens. There were 4 moisture-stored Maize (12%, 14%, 16% and 18%) which had been stored for 8 months and fed to broilers. 360 4-day-old AA+ male broilers were randomly divided into 4 treatments (6 replicates with 15 birds each) fed basic diet. At the age of 14 to 18 days, 3 broilers in every replicate were fed by metabolic diet. Maize properties, broiler performance, the apparent digestibility of nutrient and intestinal histomorphology were tested. The results showed that the soluble pentosan, resistant starch, in vitro viscosity and the activities of beta-amylase of maize decreased with the increasing of stored-moisture content. From d 4 to d 14 the high moisture-stored Maize had a tendency to increase ADFI and FCR ($P < 0.10$). The digestibility of energy, organic matter, phosphorus and total starch increased with the increasing of storage moisture linCorncobsly ($P < 0.05$). The relative weight of proventriculus decreased with the increasing of storage moisture content linCorncobsly ($P < 0.05$), meanwhile stored-moisture had a tendency to improve the relative weight of duodenum, ileum and caecum quadratically ($P < 0.10$). The result suggested that high moisture storage could help to decrease the anti-nutritional factors and improve the nutritional value of Maize. Increasing storage moisture could improve the performance for broilers and increase the utilization of nutrient and the development of gastrointestinal tracts.

Keywords: stored-moisture, Maize, broilers, anti-nutritional factors, nutritional value

S1-0154 The impact of diets supplemented with medium chain fatty acids on the performance of broilers challenged with clostridium perfringens

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The inclusion of medium chain fatty acids (Aromabiotic®) in broiler diets has demonstrated antimicrobial effects. A 42-day, 30 bird/pen, 4 treatment, 10 replications broiler study was conducted to compare the performance and lesion scores of straight-run Cobb 500 broiler chicks, vaccinated with Coccivac® and challenged with *Clostridium perfringens* on Day 17 (a titrated *Cl. perfringens* culture for a mortality of 5-10 % was inoculated to the birds). Dietary treatments were: 1) negative control (no additives); 2) positive antibiotic control (55 g/MT BMD® in starter and grower and 22 g/MT STAFAC® in finisher); 3) Aromabiotic® FULL dosage (AB FULL) (1.8 kg/MT in starter, 1.4 kg/MT in grower, 0.9 kg/MT in finisher); 4) Aromabiotic® HALF dosage (AB HALF) (0.9 kg/MT in starter, 0.7 kg/MT in grower, 0.5 kg/MT in finisher). Day 21 necrotic enteritis lesion scores for the positive control and AB FULL were similar ($P > 0.05$), but lower ($P < 0.05$) than the negative control, whereas AB HALF was similar ($P > 0.05$) to the negative control. Day 42 body weights for AB FULL and AB HALF were similar ($P > 0.05$) to the positive control and greater ($P < 0.05$) than negative control. AB FULL and the positive control had improved ($P < 0.05$) 0-42 day mortality-adjusted FCR compared to negative control. Day 0-42 mortality percentage were lower ($P < 0.05$) for each of the additive treatments (range 3.79 to 2.62%) compared to the negative control (9.01%). The European Poultry Efficiency Factor Day 42 scores (range 234.7 to 245.7) for all additive treatments were similar ($P > 0.05$) and higher ($P < 0.05$) than the negative control (197.9). For this study, Aromabiotic® was comparable to the AGP feeding program of BMD® in the starter and grower feeds shuttled to STAFAC® in the finisher feeds in supporting live performance and reducing necrotic enteritis lesion scores of broilers that were vaccinated for coccidiosis, exposed to used litter, and challenged with *Cl. perfringens* in a litter floor pens trial.

Keywords: medium chain fatty acids, clostridium perfringens, necrotic enteritis, Aromabiotic®

S1- 0155 Riboflavin deficiency impairs liver fatty acid oxidation in starter Pekin ducks

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The objective of this study was to investigate the effect of riboflavin deficiency on growth, liver lipid metabolism, and the underlying mechanism in starter Pekin ducks. A total of 360 one-day-old Pekin ducks were divided into three groups of 120 birds each, with 12 replicates and 10 birds in each replicate. The ducks in 2 of 3 groups had free access to a riboflavin-deficiency diet (RD group) or a control diet (C group) for 21 days. The birds in third group (PF group) were pair-fed the control diet to the mean intake of the RD group. Body weight gain and gain: feed were lower in the RD group than the C and PF groups, as well as lower riboflavin content in plasma and liver. Increased liver relative weight, lipid accumulation in liver and plasma were observed in riboflavin-deficient ducks. The isobaric tags for relative and absolute quantification (iTRAQ) based quantitative proteomics approach was performed to explore the liver proteome altered by riboflavin deficiency. Flavoproteins, such as acyl-CoA dehydrogenase family (ACADS, ACADM, ACAD9), were all down-regulated in the RD group, indicating liver fatty acid oxidation impaired in response to riboflavin deficiency. In conclusion, fatty acids oxidation impairment in liver leading to lipid accumulation, increased liver relative weight, and liver damage may be the major mechanisms responsible for reduced growth performance of riboflavin-deficient ducks, as well as poor riboflavin status.

Keywords: riboflavin, deficiency, lipid metabolism, proteomics

S1- 0156 Feeding laying hens a low protein diet with subsequent waste water treatment in the effort of nitrogen and ammonia mitigation

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The present study was aimed at evaluating whether lowering crude protein (CP) diet could reduce excreta nitrogen (N) and ammonia (NH₃) volatilization in laying hens without impacting egg quality. Two experimental diets containing 17 (control) and 15% (low) CP were assigned to two groups of 22-week-old ISA Brown hens, so each treatment had 20 replicates with 6 to 7 birds each. Manure dropped on the floor from each dietary group was drained out to two 4-terraced ponds in which aquatic plant (*Azolla pinnata*) was grown (in the last pond level) to filter the waste (manure) water. *Azolla* was also grown in other 4 ponds with regular water as control. Lowering the CP to 15% resulted in lowered excreta NH₃ by 21.8% ($P \leq 0.05$) although it did not alter N content and microbial counts. Dietary CP, the presence of *Azolla* in the pond, or dietary CP by *Azolla* interaction had no effect on the total dissolved solids (TDS) of the waste water. There was no difference in N contents between *Azolla* biomass in the two dietary groups, and between the two dietary groups and the control. Eggs laid by the 15% CP hens had comparable quality with those by the 17% CP counterpart. The study suggested that reducing dietary CP from 17 to 15% helped lower NH₃ volatilization and had no negative effect on egg quality. Growing *Azolla* in the pond failed to elicit a clear filtering effect.

Keywords: ammonia, nitrogen, hen, low protein, egg quality, waste water quality

S1-0157 How reliable is your nutritional database: erroneous sources may cause significant underestimation of ingredient AME values

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Apparent metabolisable energy (AME) bioassay as the assessment of the energy in the dietary ingredients available to animals is a preferred system for feed formulation in poultry. Largely, the AME values used in industrial or research scales are from a collection of information available in literature or of values assayed individually in the companies or institutes. Although energy is regarded as one of the key tenets of least cost diet formulation, it is still questionable that nutritionists have high confidence in their energy values of ingredients. Majority, if not all of, nutritionists actually realise that AME system is imperfect and errors occur in one way or another. These may be produced from nutritional imbalance of diets, due to the variations of energy level in standard (or known) ingredients, or by the use of inappropriate equations during calculations. In the current review of the methodologies used for AME measurements, the focus was to assess in what extent the errors are present in the published AME values for poultry and how the bioassay can be improved to more accurately measure AME of individual feed ingredients used in poultry feed formulation. In a study, for example, the erroneous equation used in the widely applied practical diet replacement assay led to more than 5% underestimation of the AME value for the ingredient under investigation. This converts to more than 150 kcal energy underestimated, which is substantial in feed formulation. Overall, the meta-analysis suggested that the underestimation of poultry feed ingredients could be between 150 – 500 kcal/kg of ingredients for broilers and layers depending on how the assays were implemented. This may converted to marked economic losses for commercial poultry production due to the energy wastage applied by such underestimation of ingredient AME values in feed formulation. A more effective and accurate assay system is required for AME measurement in poultry.

Keywords: apparent metabolisable energy, feed formulation, bioassay, poultry

S1-0159 Manipulating the nutritional profile and digestibility of grain legumes by extrusion cooking

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Extrusion cooking results in molecular and chemical transformations of feed material, hence its effect on the nutritional composition and in vitro protein and starch digestibility of Pigeon pea (*Cajanus cajan*), African yam beans (*Sphenostylis stenocarpa*) and Bambara groundnut (*Vigna subterranea*) was evaluated in this study. Pigeon pea (PP), African yam beans (AY) and Bambara groundnut (BG) were extrusion cooked in a single screw extruder under two temperatures (100, 140°C), 25% moisture and 60 rpm screw speed. Extrudates were oven dried at 75°C and analyzed for crude protein (CP), crude fibre (CF), ether extract (EE), ash and total starch (TS). Digestion was simulated by incubation in buffered pepsin, α -amylase, pancreatin and amyloglucosidase at 37°C. In vitro starch digestibility (IVSD) was measured as glucose release from starch up to 180 mins post-incubation by glucometry. Hydrolysis index (HI) and glycaemic index (GI) were calculated. In vitro protein digestibility (IVPD) was estimated from nitrogen in the digestion filtrate. All analyses were performed in quadruplicates. Data was analyzed using ANOVA, means separated by LSD and statistically significant differences defined as $P < 0.05$. IVSD increased significantly for AY; 44.8, 288.2, 290.0 mg.dL⁻¹.100mg⁻¹, BG; 38.4, 275.2, 313.8 mg.dL⁻¹.100mg⁻¹, and PP; 36.6, 197.2, 267.4 mg.dL⁻¹.100mg⁻¹ for raw, 100°C and 140°C extrudates respectively. Similar trend was recorded for HI and GI. IVPD also increased ($P < 0.05$), 26.0, 65.3, 85.4% for raw, 100°C and 140°C extrudates of AY respectively. Extrusion cooking did not however influence IVPD for BG ($P > 0.05$). Higher IVPD was recorded for PP at 100°C (84.4%) compared to 51.2 and 61.9% for raw and 140°C extrudates of PP. Extrusion cooking lowered EE, Ash and CP, and increased CF for all samples. Extrusion of these grain legumes could improve their use as energy and protein ingredients in monogastric feeding.

Keywords: extrusion cooking, in vitro starch digestibility, in vitro protein digestibility, grain legumes, glycaemic index

S1-0161 Effect of dietary fiber levels on growth performance and ileal digestibility of amino acids of meat duck

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A study was conducted to determine the effects of dietary fiber levels on growth performance and the apparent (AID) and standardized (SID) ileal digestibility of amino acids in ducks from d 1 to 35. One-day-old Cherry Valley ducks were fed diets containing different levels of crude fiber (CF, 1.46%, 3.09%, 4.15%, 6.18%, 7.52%, and 9.03%, based on analysis) from 1 to 21 d of age. After that, ducks during 22 to 35 d of age were fed the one diet (4% CF) during different treatments to examine the recovery growth of ducks. Each dietary treatment consisted of 8 replicate pens of 15 birds. All data were analyzed using the one-way ANOVA, Linear and quadratic regression procedure of SAS 8.0. Body weight, body weight gain and feed to gain ratio of ducks aged from 1 to 21 d were increased ($P < 0.01$) in a quadratic manner by the increment of dietary fiber levels. Feed intake during 1 to 21 d of age linearly increased ($P < 0.01$) with the increase of dietary fiber. Ducks in each treatment fed the same diet during 22 to 35 d showed no difference in growth performance ($P > 0.05$). The AID and SID of Arg, Ile, Leu, Thr, Val, Asp, Ala, Glu, Gly, Pro, and Ser were linearly increased ($P < 0.05$) with the increase of dietary fiber, while the highest digestibility were observed in 7.52% CF diet, except for Thr and Pro (in 4.15% CF diet). These results suggested that 3.09% - 7.53% CF in diet for meat duck aged from 1 to 21 d had an improvement on growth performance and AID or SID of most amino acids.

Keywords: duck, growth performance, ileal digestibility of amino acids, dietary fiber

S1-0164 Effect of in-ovo ascorbic acid on bone strength of broiler chickens subjected to heat stress during incubation and growth

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An experiment was conducted to evaluate the effect of ascorbic acid (AA) injection on broiler bone development when subjected to heat stress during incubation and creation. One Thousand (1,000) fertile Cobb® eggs, were used. The eggs were evenly distributed by weight in five incubators (200 eggs each). The incubation treatments were: eggs without AA injection and incubated at 37.5°C; eggs without AA injection and incubated at 39°C and eggs injected in ovo of 6 µg of AA/100 µL of water prior to incubation and incubated at 39°C. During the growth phase, the birds were raised in barns at three different temperatures: cold, thermoneutral and hot. The data were analyzed using the General Linear Model procedure of the SAS® program and the means were compared by Tukey test at 5% probability. The left femur and tibia were used for the mechanical bone strength tests, to determine the maximum permissible force of the bone, amount of deformation caused by the Fmax, and the determination of bone rigidity. Significant effects ($P < 0.05$) for femur maximum strength and flexibility maximum strength were observed. Lower values were found in the birds from AA injected eggs incubated at 39°C and grown in hot temperatures. Significant effects ($P < 0.05$) were also found for the tibia maximum strength, with the lowest values found for chickens grown under hot temperatures. As respects femur and tibia rigidity: there was no effect ($P > 0.05$) for any treatment or temperature. Biomechanical parameters are direct indicators of bone quality and are determined by the bone density and the maximum flexibility strength. High incubation temperatures combined with or without pre-incubation in ovo ascorbic acid injection, did not minimize the effects of high environmental growth temperature on bone strength of broiler chickens reared until 42 days of age.

Keywords: deformation, femoral, rigidity, tibia, vitamin C

S1- 0165 Christmas bush(*Alchornea cordifolia*) in poultry nutrition: effect of some local processing methods

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Christmas Bush (*Alchornea cordifolia*) is a tropical browse plant that yields heavily in forages and fruits. It is commonly used as a medicinal plant throughout its area of distribution. The leaves are mostly used, but also the stem bark, stem pith, leafy stems, root bark, roots and fruits enter in local medicine. The leaves are also used as forages for small ruminants and leaf meal for poultry, but the fruits or seeds are little known and used as human food or animal feed. Available data on its nutritional value indicate that the fruits are high in carbohydrates, but contains toxic substances that limits its use in poultry feeds. We examined the effect of some local processing methods (soaking, boiling and soaking in water before boiling) of Christmas bush fruits and seeds on proximate compositions, anti-nutritional factors and performance of broilers in different experiments. Proximate compositions and anti-nutritional factors of the dried fruit, seed and pulp varied considerably depending on the type. The fruits, seeds and pulp contained 8.20, 15.00 and 24.01% crude protein and 0.26, 0.55 and 54.07% fat respectively. Processing of the fruits and seeds increased the fat and crude fibre contents, but decreased the ash. Crude protein and carbohydrates were unaffected. All the processes caused significant decrease in the anti-nutritional factors and totally eliminated the tannins in the seeds but not the fruits. Soaking in water was less effective in reducing the tannins content of the fruits while boiling was less effective in reducing the phytic acid contents of both fruits and seeds. Soaking, fermentation and boiling of the dried fruit or fruit meal, allowed for only 10% inclusion in broiler diets. This paper discusses various methods used in improving the nutritive value of Christmas bush fruits and/or seeds for poultry and calls for further research into the use of the pure seed in poultry nutrition considering its crude protein and fat values.

Keywords: christmas bush, fruits, processing, chemical compositions, broilers, performance

S1- 0166 Effect of dietary selenium source on selenium deposition in broiler muscle tissue

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Aim of this preliminary study was to investigate the effect of different dietary selenium sources on the selenium deposition in broiler muscle tissue. For this purpose, 4 pens with 30 Ross 308 male birds each were fed from day 0 to 14 one of the 4 diets, which differed only in the added selenium source. Treatment 1 contained no added Se. Diets of treatments 2, 3 and 4 were supplemented with 0.2 mg/kg Se from respectively sodium selenite (NaSe), L- selenomethionine (SeMet) and seleno- hydroxy- methionine (SeOH). Samples of breast meat (whole left fillet) were taken from 6 birds from each treatment on day 7 and also from 6 birds on day 14. Initial Se level in breast meat was determined on 6 birds euthanized on day 0. Breast meat samples were analyzed for selenium content by HLPC ICP-MS. Se content of broiler muscle tissue on day 0 was 197 ± 10 µg/kg. On day 7 the Se content of the non- supplemented birds decreased and reached a level of 84 ± 6 µg/kg. Birds fed supplementary Se as sodium selenite also had a decreased Se (130 ± 7 µg/kg) on day 7. However, treatment with SeOH and SeMet resulted at day 7 in increased Se levels of 229 ± 14 and 269 ± 24 µg/kg, respectively. On day 14, the Se content in muscle tissue was 68 ± 2 µg/kg in non-supplemented diets and 121 ± 6 , 240 ± 12 and 271 ± 28 µg/kg for NaSe, SeOH and SeMet supplemented diets, respectively. Results from this trial suggest that Se deposition in broiler muscle tissue is dependent of the supplemented Se source. Supplementing inorganic Se (NaSe) results within 7 days in a decrease of Se in broiler muscle, while supplementing organic sources results in an increased level of Se in broiler muscle and these levels are stable over time. These findings provide a good indication for future research of the effect of Se sources on the selenium status in poultry.

Keywords: selenium, broiler, selenomethionine, organic selenium

S1-0167 Effects of multi-strain probiotics supplementation in diet on growth performance and intestinal microflora in native chickens

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Probiotics can beneficially affect the host by improving intestinal microflora balance. The main purpose of this study was to investigate the effect of mix probiotics on growth performance and intestinal microflora in broilers. A total of three hundred 1-day-old L2 strain Taiwan country chickens were randomly assigned into 12 pens of 25 chickens each, and every treatment had 4 replicated pens with two pens of male and female respectively. A corn-soybean meal based control diet was formulated and experimental diets included 0.1% multi-strain probiotics (0.1% *Lactobacillus acidophilus* LAP5, *L. fermentum* P2, *L. casei* L21 and *Pediococcus acidophilus* LS) and 10 ppm antibiotics (avilamycin). All groups were treated for three months. The results showed that multi-strain probiotics had no significant effect on growth performance ($P>0.05$). Compared to the control group, multi-strain probiotics had significantly increased cecum *Lactobacillus* counts as well significantly reduced cecum *Clostridium perfringens* and *Escherichia coli* number. Multi-strain probiotics treatment also increased volatile fatty acids (acetate acid and butyrate acids) and lactic acids of cecum to the control group. In conclusion multi-strains probiotics in diet had the potential to enhance probiotic counts and improve intestinal health in chickens.

Keywords: chickens, intestinal population, multi-strain probiotics

S1-0168 Digestible lysine and methionine + cystine levels on performance and breast meat quality of broilers at 42 days old

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The experiment was conducted to evaluate different levels of lysine and methionine+cystine on performance and breast meat quality of broilers at 42 days old. Were used 3200 broilers from 1 day old lineage "Cobb 500," distributed in a completely randomized design in a factorial 2X5 (2 lysine levels - 1.100 and 1.210% X 5 levels of methionine + cystine - 0.724; 0.764; 0.804; 0.844; 0.884%) and eight replicates of 40 birds each. Performance parameters evaluated were weight gain, feed consumption and feed conversion ratio. At 42 days of age, 480 birds were culled to the achievement of sampling pectoral muscle, which were submitted to analysis of the following parameters of meat quality: brightness, redness, yellowness, cooking loss, strength shear and pH. Statistical analyzes were performed by using SAS ® and in case of significant effect regression analyzes were performed. There was no effect of lysine and methionine+cystine for any of variables. In conclusion, diet containing the lowest digestible lysine (1.100%) and methionine+cystine (0.724%) level achieved the requirements of birds on performance and breast meat quality during the period from 22 to 42 days old.

Keywords: amino acid, muscle, pH, softness

S1- 0169 Discussion on the effects and gene regulatory mechanism of polysavone on cholesterol metabolism in laying hens

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The experiment aimed to investigate the effects of Polysavone on cholesterol metabolism in laying hens, and tried to elucidate its regulatory mechanisms of gene expression. A total of 540 26-week-old healthy Nick laying hens were randomly divided into 5 groups consisting of 6 replicates of 18 birds each pen. Laying hens in control group were fed with corn-soybean basal diet, and other group were provided with the basal diets supplemented with 300, 600, 900, 1200 mg/kg polysavone, respectively. The pre-test lasted for 7 days, and the experiment lasted for 70 days. On day 35 and day 70 of experiment, the yolk cholesterol content and whole egg cholesterol content were detected. At the end of experiment, the serum biochemical parameters of laying hens were measured. The mRNA expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), sterol regulatory element-binding protein 2 (SREBP-2), cholesterol 7 α -hydroxylase (CYP7A1) in liver tissue and occyte vitellogenesis receptor (OVR) in ovarian tissue were measured by real-time PCR method. The results demonstrated that on day 35 and 70, the yolk cholesterol content and the cholesterol content of whole egg in 900 mg/kg Polysavone group were significantly lower than the control group. Compared with the control group, the high density lipoprotein-cholesterol (HDL-C) in 900 mg/kg Polysavone group and the ratio of HDL-C to LDL-C in 600 and 900 mg/kg Polysavone groups significantly increased. Compared with the control group, the mRNA expression of HMGCR in 600, 900, 1200 mg/kg Polysavone groups significantly decreased, and the mRNA expression of CYP7A1 in 600 and 900 mg/kg Polysavone groups significantly increased. Therefore, dietary supplementation of Polysavone can decrease the yolk cholesterol and whole egg cholesterol content. The mechanism underlying the cholesterol-lowering is regulated mainly through reducing endogenous synthesis and promoting the excretion of cholesterol.

Keywords: polysavone, laying hen, cholesterol metabolism, gene expression

S1-0170 Effect of a monocomponent protease on the standardised ileal amino acid digestibility of feed ingredients for broiler chickens

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The influence of adding a monocomponent protease (RONOZYME ProAct; 15,000 PROT/kg diet), on top of assay diets containing a background of microbial phytase (RONOZYME HiPhos; 1,000 FTU/kg diet), on the standardised ileal amino acid digestibility in two cereals (sorghum and broken rice), five protein sources (soybean meal, rapeseed meal, guar meal, palm kernel meal and maize DDGS) and a cereal by-product (two samples of rice polishings) was determined using 3-wk-old broilers. The endogenous amino acid losses were determined following the feeding of a protein-free diet and used for the calculation of standardised coefficients. Responses to added protease varied depending on the feedstuff and the amino acid considered. Average standardised digestibility coefficients of 17 amino acids in test feedstuffs without and with the protease were: sorghum, 0.84 and 0.86 ($P < 0.10$); broken rice, 0.85 and 0.89 ($P < 0.001$); soybean meal, 0.81 and 0.83 ($P < 0.05$); rapeseed meal, 0.78 and 0.79 ($P > 0.05$); guar meal, 0.53 and 0.56 ($P < 0.10$); palm kernel meal, 0.58 and 0.60 ($P > 0.05$); maize DDGS, 0.70 and 0.71 ($P > 0.05$); rice polishings 1, 0.72 and 0.74 ($P < 0.05$) and rice polishings 2, 0.67 and 0.71 ($P < 0.001$), respectively. When individual amino acids were considered, the increments in digestibility were generally higher for threonine and this effect was consistent across most feedstuffs. These data are suggestive of the potential value of this monocomponent protease to improve protein digestibility of diets containing supplemental microbial phytase.

Keywords: amino acid, ileal digestibility, feedstuffs, protease, broilers

S1- 0171 Long- term effects of a *Buttiauxella* sp. phytase on performance, egg-shell quality, apparent ileal Ca and P digestibility, and bone properties of white egg layers

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The effects of a *Buttiauxella* sp. phytase (BP) in diets with reduced Ca and available P (avP) were studied in Lohmann LSL Lite hens from 30 to 70 weeks of age (woa). Hens [n=456 (4 birds in each of 114 cages)] were fed either a nutritionally-adequate positive control diet (PC); the PC diet with Ca and avP levels reduced by 0.134 and 0.146% units, respectively (NC); or the NC diet + BP at 250 FTU/kg (NC+BP). Egg production (EP), BW, feed efficiency, eggshell thickness and breaking strength from 30 to 70 woa, and apparent ileal Ca (AIDCa) and P (AIDP) digestibility, and femur bone density, breaking strength (BBS) and ash content at 32, 48, and 70 woa were measured. Parameters were analyzed for diet×age interaction and main effects. Diet did not affect EP (94.3% across diet and age), feed efficiency, shell thickness and breaking strength, AIDCa or bone ash. Body weight of the NC+BP (1.73±0.01 kg) and PC (1.72±0.01 kg) hens was higher (P=0.040) than the NC hens (1.69±0.01 kg) across ages. AIDP was higher in the NC+BP hens (52.7±3.38%) than in NC hens (39.9±3.38%) at 32 woa, higher in the NC+BP hens (60.7±3.38%) than in PC hens (49.6±3.61%) at 48 woa, and higher in the NC hens (54.2±3.38%) than in NC+BP hens (39.2±3.38%) at 70 woa (interaction P=0.001). BBS of NC+BP hens (20.3±0.85 kgF) tended to be higher (P=0.069) than NC hens (17.8±0.85 kgF); PC hens were intermediate (19.1±0.85 kgF) at 32 woa, with no diet effects at 48 or 70 woa. Cortical bone mineral content (CBMC) at 70 woa followed a similar pattern as observed for BBS at 32 woa. The NC hens maintained egg production and shell quality at the expense of BW and bone quality. The greater AIDP and BBS at 32 woa, CBMC at 70 woa, and BW across age in the NC+BP hens than the NC hens and the non-difference from the PC hens showed that BP at 250 FTU/kg can replace 0.134% Ca and 0.146% AvP, increased age-specific dietary P availability and supported long-term maintenance of structural bone in white egg laying hens.

Keywords: *Buttiauxella* sp. phytase, layers, available P, bone, calcium, digestibility

S1- 0174 Graded inclusion of white lupin (*Lupinus albus*) meal depress laying hens performance and nutrients digestibility

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In last years, interests in the use of native legume seeds as protein components in diets for monogastric animals increased again. The aim of the study was to determine the usefulness of white lupine (WL) in laying hen diets and its influence on birds' performance and egg weight. The experiment was conducted with 360 layer hens Hy Line Brown located in cages (3 birds/cage). The birds were randomly assigned to six treatments, each with 60 hens and during the period of 17 weeks, they were fed diets with increasing lupine meal content - 0, 6, 12, 18, 24 and 30% . The environmental conditions were managed according to the standard requirements for Hy Line Brown layer hens. All diets were formulated to be isonitrogenous and isocaloric. The body weight, laying rate, egg weight, feed intake and feed conversion were registered. There was no negative effect of white lupine meal inclusion on feed intake during whole trial. The mean value of feed intake for 17 weeks amounted to 115g per hen/day. In the experiment, a decrease in laying rate was recorded in treatments where 24 and 30% of WL was used. The mean value of laying rate for 17 weeks amounted to 95.4 (0- 18% of WL), 90.7 (24% of WL) and 87.9 % (30% of WL) (P<0.01). The egg weight was diversified already after 7 weeks of egg production and was, on average: 58.5 (0, 6, 12 % of WL), 56.2 (18, 24 and 30% of WL) g (P<0.01). High concentration of lupine meal had a negative effect on FCR. The mean value of FCR for 17 weeks amounted to 2.14 (0 and 6 % of WL), 2.25 (18 and 24% of WL) and 2.37 (30% WL) kg/kg egg weight (P<0.01). Apparent ileal digestibility of dry matter, ether extract, crude protein and starch, linearly decreased (P< 0.05) as WLM increased from 0 to 300 g/kg. There was a quadratic effect (P< 0.05) of WLM dose on sialic acid excretion. It could be concluded that 18% of white lupine in layer hens diet could be used as a valuable protein source, without negative effect on laying rate and feed intake.

Keywords: digestibility, viscosity, laying hen

S1-0175 The nutritional value of yellow lupin (*Lupinus luteus*) for broilers

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It is well known that nutrient composition and anti-nutritional factors in yellow lupin, generally, depend on the cultivar and growing conditions. Yellow lupine seeds, 7 varieties were harvested in 2013. The experiment was conducted with 240, 16-day old Ross 308 male chickens. The reared birds were randomly located in cages and allotted 7 dietary treatments (10 replications in each, three birds per replication). For seven days, birds were fed experimental diets (30% - yellow lupine seeds) and basal diet (70% - maize-soybean). On day 20 and 21, excreta were individually collected twice per day and immediately frozen. On the 22nd day of the experiment, 30 chickens from each group were sacrificed and the ileum was removed. To allow digestibility to be determined, 3 g/kg TiO₂ was included as a marker. Apparent ileal digestibility (AID) was calculated using the difference method. Soluble NSP ranged from 24 to 95 mg/g (as-is basis). There were differences ($P<0.05$) in ileal digesta viscosity (4.97 to 8.61 mPas · s) and feed intake (115 - 121 g/d/bird) between yellow lupine cultivars. The obtained correlation coefficients (r) for raffinose content explained over 85% DM AID variability ($r = -0.85$, $P<0.05$) and 58% ether extract AID variability ($r = -0.58$, $P<0.05$). The relationship between soluble NSP content and the ether extract AID ($r=0.26$) was linear ($P<0.05$). Sialic acid excretion was positively correlated with raffinose content ($r=0.38$). The obtained correlation coefficient (r) for raffinose content explained over 67 % of variability in AMEN of yellow lupine seeds ($r = -0.67$, $p<0.05$). The correlation coefficient for soluble NSP content tend to explain 26 % of variability in ether extract apparent ileal digestibility ($r = -0.26$, $P<0.06$). It seems that the nutritional value of yellow lupin seeds for broilers depends, to a considerable extent, on raffinose content and ileal viscosity (soluble NSP content).

Keywords: yellow lupin, broiler chickens, raffinose, NSP, viscosity

S1- 0176 Effect of a sodium butyrate- additive on broiler performance and nutrient use

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The objective of this study was to evaluate the effect of a sodium butyrate-based additive on broilers performance, jejunal morphology and diet digestibility when energy and amino acids concentrations were reduced. One-day-old male Ross 708 broiler chicks were fed dietary treatments in a 3 x 2 factorial design (6 treatments; 8 pens/treatment) with 3 dietary formulations (control diet, control diet formulated with a reduction of 2.3% of amino acids and 60 kcal/kg, and control diet formulated with a reduction of 4.6% of amino acids and 120 kcal/kg) with or without the inclusion of vegetable fat protected sodium butyrate (SB). Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were recorded. At 28 d of age the ileal digestibility of dry matter, energy and protein were evaluated, as well as jejunal morphology. The model for the statistical analysis included the main effect of diet, SB and its interaction (SAS 9.4). At 14 and 28 d of age, the SB improved the BWG ($P<0.05$), without affecting the FCR. Considering the entire experimental period (1-42 d), the amino acid and energy reduction impaired BWG by 6% ($P<0.01$), while the SB improved BWG by 2% ($P<0.05$). Analyzing the interaction, the first level of energy/amino acid reduction supplemented with SB led to the same BWG observed in the control treatment. The nutrient reduction impaired FCR by 5% ($P<0.01$), and no effect of SB supplementation nor interaction was observed. No differences were observed for villus height, crypt depth, villus: crypt ratio and number of goblet cells. The SB supplementation decreased the energy, protein and dry matter digestibility by 3, 2 and 2% respectively. Based on our findings, SB supplementation was able to recover the reduction in BWG due to the dietary reduction of 2.3% of amino acid and 60 kcal of energy, without affecting the jejunal morphology. Nevertheless, more studies are necessary to understand the mechanism of action in which this product enhances the BWG.

Keywords: sodium butyrate, broilers, nutrient reduction.

S1- 0177 Evaluation of soy protein concentrate and spray dried porcine plasma at two different amino acid concentrations in prestarter diets for performance of broiler chickens at market age

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Feeding broiler chickens with specialized prestarter diets is gaining importance as the first week live weight has been shown to be positively correlated to body weight at the end of production cycle. The efficiency of inclusion of two main protein sources; soybean meal (SBM) or soy protein concentrate (SPC), sprayed dried porcine plasma (SDPP) (0 and 2%) and amino acid (AA) concentration (normal or 10% extra) were examined in prestarter diets fed to broilers using a $2 \times 2 \times 2$ factorial arrangement of treatments. A total of 576 day-old male Ross broiler chicks were assigned to the 8 treatments, each replicated 6 times with 12 birds per replicate in 48 floor pens with hardwood shavings. Experimental treatments were fed for the first 10 days of age with common grower (d 10-24) and finisher (d 24-35) diets given. Diets were formulated to be isoenergetic. For the amino acid concentration, normal and 10% higher digestible essential AA above the recommendations for Ross 308 broilers were formulated. Feeding 10% higher AA increased ($P < 0.05$) feed intake of birds during the first 10 days and remained significant to the end of study at d 35 ($P < 0.01$). Inclusion of SDPP reduced feed intake ($P < 0.01$) and body weight gain (BWG) ($P < 0.001$) in the starter phase with no carry over effect for subsequent phases of growth. Compared to SBM, inclusion of SPC decreased ($P < 0.01$) BWG and FCR until day 10. There was a significant interaction between SDPP and AA level where the reduction of BWG by feeding SDDP was only observed when diets contained lower AA. Feed conversion ratio (FCR) was improved only until day 10 in birds fed 10% higher AA. Overall FCR (d 0 to 35) was not affected by the experimental treatments. The results showed the distinctive positive effect of increasing AA concentration in prestarter diets with carry over effect for the body weight at market age. Conversely, no positive lasting effect of SDPP or SPC was observed for growth performance of broiler chickens.

Keywords: prestarter diets, carryover effect, soy protein concentrate, plasma protein

S1-0179 Effect of calcium, phosphorus and multi-vitamin on the performance of Dahlen meat-quality hens from the beginning laying to the peak laying period

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In the present study, we use the $2 \times 2 \times 2$ factorial design to investigate the effect of different levels of calcium (3.5%, 3%), non-phytic acid phosphorus (0.3%, 0.25%) and multi-vitamin for breeder hen (300g/T, 400g/T) (Hualuo Co.) in the corn-soybean diet with supplementation of phytase (300U/kg) on the egg production and eggshell quality in Dahlen meat-quality hens. In the present study, we aim to determine the best proportion of calcium, phosphorus and multi-vitamin in the diet to decrease the broken egg ratio. One thousand six hundreds 22-weeks old S06 healthy hens with similar body mass were selected and assigned randomly to different treatments, 10 repetitions for each treatment, and 20 hens for each repetition. Preliminary test was conducted for 1 week and the formal test was conducted for 10 weeks. The result showed that in 3% calcium with 0.3% phosphorus group, there was significant increases in egg production during the peak period while significant decreases in the broken egg ratio during the late period, however, there was no significant difference in egg weight, broken egg ratio, eggshell thickness, eggshell strength, soft shell egg ratio and malformation egg ratio among different groups. There was no significant effect of the multi-vitamin on egg production and broken egg ratio. These results suggested that for 23-33 weeks-old Dahlen meat-quality hens, the suitable proportion of the calcium, non-phytic acid phosphorus and multi-vitamin in the corn-soybean diet with supplementation of phytase is 3%, 0.3% and 300g/T, respectively.

Keywords: calcium, non-phytic acid phosphorus, multi-vitamin, Dahlen meat-quality hens, egg production, broken egg ratio

S1-0180 A novel SNP in Z chromosome is associated with chicken early/late feathering

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Early and late feathering phenotype provides a convenient and harmless approach to gender identification of chicks at hatch. The molecular architecture of the sex-linked early/late feathering trait was identified as the insertion of the endogenous retrovirus 21 (ev21) or the partial duplication of the PRLR and SPEF2 genes. However, the identified causal mutation had poor detection efficiency in some strains with early/late feathering trait in our company. Therefore, we carried out this study to detect new causative mutations which are suitable for these strains. We re-sequenced the mutation region ranging from 10.56Mb to 11Mb in Z chromosome of chickens with early and late feathering phenotype respectively and captured two novel SNPs that were closely linked with feathering traits, both of which were T locus linked with early feathering phenotype and C locus linked with late feathering phenotype. Then we validated the two SNPs in 831 samples from seven strains (four brown eggshell strains and three white eggshell strains) by PCR- restriction enzyme digestion method. Results showed that one of the SNPs had high linkage relation with early/late feathering phenotype. And the accuracy of this method was proved to be as high as 99.9% in auto-sexing populations through genotyping blood without any feathering information from randomly selected chickens. These results showed that the novel SNP was feasible to identify the genotype of chicks with early/late feathering phenotype for flock purification in our strains.

Keywords: early and late feathering phenotype, sexing, chick, SNP

S1-0181 Influence of dietary metabolizable energy of ducks exposed cold stress

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This study was carried out to investigate the effects of dietary metabolizable energy of ducks exposed cold stress on the growth performance, blood parameter, corticosterone in serum. A total of 500, 21-d-old Pekin Ducks (initial BW=1,089 \pm 5.21 g) allotted to five dietary treatments of 2, 950, 3,000, 3,050, 3,100, and 3,150 kcal MEN/kg (calculated, as-is basis) with 4 replicate pens and 25 ducks per pen in a 21-d feeding experiment. All Ducks were floor pens in an environmentally controlled room maintained at 8°C. During the experiment, hens were provided with feed and water ad libitum. Increasing inclusion level of MEN in diets increased final body weight and body weight gain (linear, $P<0.01$). Feed intake was decreased (linear, $P<0.01$) with increasing inclusion level of MEN and improved feed conversion ratio (linear, $P<0.01$) was observed as inclusion level of MEN in diets increased. Heterophil decreased (quadratic, $P<0.05$) with increasing inclusion level of MEN in diets and increased lymphocyte (linear, $P<0.01$) was observed as inclusion level of MEN in diets increased. H:L ratio was increased (linear, $P<0.01$) with increasing inclusion level of MEN in diets. Increasing inclusion level of MEN in diets decreased (linear, $P<0.01$) corticosterone values in serum at 2, 14, and 21 d of the exposed cold stress. A similar result of decreased (linear, $P<0.05$) corticosterone values in serum at 7 d of the exposed cold stress was observed. In conclusion, feeding diet containing increasing concentrations of MEN in diets of 3,150 kcal/kg to improves growth performance, heterophils, H:L ratio, and corticosterone in serum of ducks.

Keywords: cold stress, corticosterone, ducks, metabolizable energy, H:L ratio

S1-0183 Evaluation of growth performance and intestinal microflora using probiotics supplemented in broiler diets

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This study was conducted to application of probiotic, *Bacillus amylaliquefaciens* (BA) and *Saccharomyces cerevisiae* (SC) as feed additive to evaluate the effects of growth performance and intestinal microflora in broilers. Four hundred one-day-old broiler chickens were randomly divided into 16 pens of 25 chickens each, and every treatment had 4 replicated pens with two pens of males and females respectively. Control diets (corn-soybean meal) and experimental diets, including 0.1 % BA, the mixture of 0.05 % BA and 0.05 % SC, and 10 ppm avilamycin antibiotic, were fed during 5 weeks. Results showed no significant difference in the growth performance among all groups. Added of the mixture of BA and SC increased acetate and propionate and decreased the *E. coli* in ceca compared to control and antibiotic treatments. Additionally, addition of BA and the mixture of BA and SC compared to antibiotic treatment decreased serum glutamic-oxalocetic transaminase and increased serum high-density lipoprotein. In conclusion, supplementation of the mixture of BA and SC was greater than added BA only, and the mixed probiotics product could potentially alter the use of avilamycin in diets in chickens.

Keywords: probiotics bacillus; broilers

S1-0184 Effects of digestible dietary threonine levels on laying performance, serum free amino acids and biochemical indices of laying hens fed low crude protein diets during the peak production period

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The aim of this study was to evaluate the effects of digestible dietary threonine (Thr) levels in the low protein diet on laying performance, egg quality, serum free amino acids and biochemical indices of laying hens during the peak production period. Heavy Roman Brown laying hens ($n=480$), 28 wk of age, were allocated to 6 dietary treatments groups, each of which included 5 replicates of 16 hens per replicate. Dietary CP 161.8 g/kg diet was offered as the positive control group. Graded levels of dietary L-Thr were supplemented to the low crude protein diet (negative control group; 141.6 g CP) for 12-wk. Digestible Thr values are 0.43, 0.41, 0.48, 0.56, 0.65, and 0.74%, respectively. Results showed that, a reduction in crude protein content from 161.8 to 141.6 g/kg diet was without negative effects on laying performance. In the low protein diet, increasing digestible dietary Thr to 0.56 or 0.65% increased ($P<0.05$) egg production and egg mass. Interior egg quality, as indicated by Haugh units and albumen, was not significantly influenced by dietary treatments. Serum total protein, albumin, alkaline phosphatase, glucose, Ca, and P concentration were not affected by supplementing L-Thr. However, serum levels of uric acid and urea nitrogen decreased ($P<0.05$) when hens fed 0.56 to 0.65% L-Thr. Concentrations of free amino acids in circulation are indicators to a combination of supply by the feed and removal for tissue use. In the current study, serum free Thr maximized ($P<0.05$) between 0.65 and 0.74% dietary L-Thr levels. However, others free amino acids did not change due to L-Thr supplementation, indicating that others amino acids were sufficient in the diet. In summary, the low crude protein diet result in optimal results of laying hens produced an average of 61 g daily egg mass with 0.65% digestible dietary Thr, corresponding to 776 mg Thr/hen/day during the peak production period.

Keywords: laying hens, L-threonine, laying performance, egg quality, free amino acids

S1-0185 Estimation of L-threonine requirement for Xinyang green shell laying hens from 32 to 42 weeks of age

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The aim of this study was to evaluate the optimal L-Threonine (L-Thr) requirement by determining the effects of gradual levels of L-Thr on laying performance, egg quality, serum biochemical indices, and immune function in Xinyang green shell laying hens. A total of 720 laying hens, 31wk of age, were allocated to 5 dietary treatment groups, each of which included 6 replicates of 24 hens. The control group was given a basal corn-peanut-soybean meal diet containing 0.47% Thr. L-Thr levels of the experimental groups were 0.57%, 0.67%, 0.77% and 0.87%, respectively. This study lasted 12 weeks and the hens were allowed a 7 d acclimation period. No significant differences were found in average daily feed intake (ADFI) or egg weight. However, egg production and feed conversion rate (FCR) response to supplemental L-Thr were quadratic ($P<0.05$), and the response was maximized at 0.57% L-Thr. Hens fed 0.57%, 0.67%, or 0.77% L-Thr showed significant higher albumen (ALB) height and haugh unit as compared with those of the other two groups ($P<0.05$). Dietary L-Thr at 0.77% resulted in linearly increasing levels of immunoglobulin (Ig) A, IgM and IgG in serum and jejunum ($P<0.05$). Serum ALB, alkline phosphatase (AKP) and uric acid (UA) content increased quadratically as supplemental L-Thr increased, and the response was maximized between 0.57% and 0.67% L-Thr. Dietary 0.67% L-Thr significantly increased ($P<0.05$) serum triiodothyronine (T3) concentration as compared with that of the control group. The results indicated that the addition of appropriate levels of L-Thr could enhance laying performance, egg quality, and immune function of green shell laying hens. According to the broken-line regression analysis based on FCR and Egg production, the optimal dietary level of L-Thr for green shell laying hens (31 to 43 wk) were 0.57% and 0.58%, respectively.

Keywords: laying hens, L-threonine, laying performance, egg quality, immunity

S1-0186 Estimation of lysine requirement for Xinyang green shell laying hens from 31 to 43 weeks of age

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This study was conducted to estimate the optimal lysine (Lys) requirement by determining the effects of gradual levels of Lys on laying performance, apparent metabolic ratio of amino acid, antioxidant enzymes activities, and immune function in Xinyang green shell laying hens. A total of 720 Xinyang green shell laying hens, 30 wk of age, were randomly allocated to 6 treatment groups, each of which included 5 replicates of 24 hens. Lysine was added to basal diet to obtain the Lys level at 0.60%, 0.65%, 0.70%, 0.75%, 0.80% and 0.85%, respectively. This study lasted 12 weeks and the hens were allowed a 7 d acclimation period. Results showed that, the laying rates in 0.60% or 0.85% Lys group were significantly lower than those of other group ($P<0.05$), while the feed to egg ratio in 0.75% Lys group was the lowest as compared to other groups ($P<0.05$). The apparent metabolic ratio of cystine in 0.70% Lys group was the highest among all the groups ($P<0.05$). The apparent metabolic ratio of lysine in 0.65% or 0.70% Lys group was higher than that in 0.85% Lys group ($P<0.05$). Hens fed 0.75% Lys had higher serum and liver total superoxide dismutase (T-SOD) activities than those of hens receiving 0.60% Lys ($P<0.05$). The concentrations of malonaldehyde (MDA) in serum and liver in 0.75% Lys group were the lowest among all the groups ($P<0.05$). Serum immunoglobulin G (IgG) level increased significantly at 0.65% Lys as compared to the 0.85% Lys group ($P<0.05$). The results indicated that the addition of appropriate levels of Lys could enhance laying performance, antioxidant and immune function of green shell laying hens. According to the quadratic regression analysis based on laying rate and feed egg ratio, the optimal dietary levels of Lys for green shell laying hens (31 to 43 wk) were 0.70% and 0.73%, respectively.

Keywords: lysine, laying performance, requirement, green shell laying hens

S1- 0187 Effects of dietary tryptophan on laying performance, antioxidative enzymes activities, and immune function in Xinyang green-shell laying hens

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The objective of our study was to investigate the effects of dietary L- tryptophan (L-Trp) on laying performance, antioxidative activity, and immune function in Xinyang green-shell laying hens. A total of 525 Xinyang green-shell laying hens (28-week-age) were randomly allocated into 5 groups, each of which included 5 replicates of 21 hens per replicate. The control group was given a basal corn-soybean meal diet containing 0.15% Trp. L-Trp levels of the experimental groups were 0.17% , 0.19% , 0.21% and 0.23% , respectively. This study lasted 12 weeks and the hens were allowed a 7 d acclimation period. Results showed that, hens fed 0.21% to 0.23% and 0.19% to 0.21% Trp had significantly higher ($P<0.05$) laying rate and average egg weight, respectively. The feed to egg ratio in 0.21% Trp group decreased remarkably as compared to that of the control group ($P<0.05$). Furthermore, dietary Trp at 0.19% resulted in linearly ($P<0.05$) increasing levels of albumen height, haugh unit, and yolk to egg ratio. Glutathione peroxidase (GSH-Px) activity was enhanced ($P<0.05$) by dietary 0.17% to 0.19% Trp in serum and 0.19% to 0.21% Trp in kidney, respectively. Supplementing Trp to the control group elevated ($P<0.05$) total superoxide dismutase (T-SOD) activity in serum, liver, and kidney, total antioxidant capacity (T-AOC) in serum and liver, and repressed ($P<0.05$) malondialdehyde (MDA) levels in serum and kidney. Moreover, serum immunoglobulin A (IgA) and complement 3 (C3) levels were significantly increased at 0.23% dietary Trp ($P<0.05$) as compared with others group. Our results indicated that dietary Trp supplementation could improve laying performance, antioxidative activity and immune function of green-shell laying hens. According to the quadratic regression analysis based on the feed to egg ratio, laying rate, and albumen height, the optimal tryptophan level in corn-soybean meal diet of Xinyang green shell laying hens (29~40 weeks) was 0.19%~0.22%.

Keywords: laying hens, tryptophan, laying performance, antioxidative, immune function

S1- 0188 Effect of dietary n- 6/n- 3 fatty acids ratio and Vitamin E level on the morphology and DNA damage of epithelial cells in the duodenum of broiler chickens

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Broiler meat enriched with polyunsaturated fatty acids (PUFAn-3) may be cheap and widely available source of PUFAn-3 in human diet. However, PUFAn-3 are susceptible to oxidation which may lead to modification of cell membrane-lipids and a tissue damage. Duodenum as the first site of lipid digestion and absorption can be particularly exposed to damage by oxidation. The effect of dietary PUFAn-3 and Vitamin E (VE) levels on the morphology of duodenum of broilers was investigated in this study. Total of 96 one-day-old Ross 308 female broilers were assigned to 4 groups, 24 birds in each, and fed 4 soybean-maize and wheat-based diets. Diets contained either corn oil (PUFAn-6/n-3>50) or a mixture of linseed and fish oil (PUFAn-6/n-3<1). Each diet was fortified either with 50 or 300 mg vE/kg. At day 9 of age 8 chickens per group were culled and middle duodenum was sampled for morphometric assessments. At day 17 and 43 six chickens per group were culled, the epithelial cells from the duodenum were isolated and their DNA damage was measured by comet assay. The villus height, crypt depth and mucosa thickness in the duodenum were lower ($P<0.03$) in chickens fed low vs. high PUFAn-6/n-3 diet, while the villi were wider ($P<0.04$) on high vE diets. Feeding low PUFAn-6/n-3 diet caused greater DNA damage in epithelial cells of 17- and 43-d-old chickens ($P<0.05$) compared to high level. The high dietary vE level resulted in greater DNA damage in 17- d-old chickens ($P<0.05$) but did not affect it in 43-d-old birds, compared with low vE level. Results indicated that PUFAn-3, due to the susceptibility to oxidation, may compromise morphological characteristics and may increase DNA damage of duodenal epithelium. It seems that high dietary level of vE is not sufficient to prevent negative effects of oxidation in duodenum or may even act as a pro-oxidative factor. The gradation of dietary VE level upon birds age may be more effective but this warrant further research

Keywords: n- 3 fatty acid, Vitamin E, Intestinal morphology, DNA damage, Comet assay

S1-0189 Effects of feeding low protein diets with methionine and lysine supplementation on carcass characteristics and serum profile of broilers

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This experiment was conducted to investigate the effects of low protein diets supplement with methionine and lysine on carcass characteristics and serum profile of broilers. 200 one-day-old Arbor Acres broilers were assigned to four groups with 5 replicates of 10 chicks. The basal diet was formulated based on NRC (1994). The experimental diets reduced 3% crude protein by the standard. The control broilers feed basal diets, the experimental diets were A, B and C, in 1 to 21 days broilers fed diets with 0.1% DLM (DL-Methionine), 0.22% Lys (L-lysine) and 0.1% DLM + 0.22% Lys supplementation, in 22 to 42 days broilers fed diets with 0.1% DLM, 0.26% Lys and 0.1% DLM + 0.26% Lys supplementation. At 21d and 42d of age, 10 birds per treatment were sacrificed and samples of breast muscle, thigh muscle, abdominal fat and liver were collected and measured. Blood samples were collected from 10 birds randomly chosen from each treatment. The results showed as follows: At 21d of age, broilers fed low protein diets with 0.1% DLM + 0.22% Lys had highest breast weight ($P < 0.05$). The highest abdominal fat weight and abdominal fat ratio were found at 0.22% Lys level ($P < 0.05$). At 42d of age, broilers fed low protein diets supplemented with 0.1% DLM + 0.26% Lys had highest thigh weight, thigh ratio and liver weight ($P < 0.05$). The serum profile results showed as: at 21d of age, low protein diets with 0.22% Lys extremely significant increased the concentration of uric acid in serum of broilers ($P < 0.01$). Supplementation with 0.1% DLM + 0.22% Lys in low protein diets significantly increased the concentrations of total protein and globulin in serum of broilers ($P < 0.05$). Broilers fed low protein diets with 0.1% DLM + 0.22% Lys supplementation increased the concentrations of TP and GLOB at 42 days of old ($P < 0.05$). The current study suggests that low protein diets supplemented with methionine and lysine improved carcass characteristics and serum profile in broilers efficiently.

Keywords: low protein diets, broilers, methionine and lysine, carcass characteristics, serum profile

S1-0190 Growth performance and egg production of Japanese quails fed diets containing graded levels of sun-dried cassava peel meal

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360 two weeks old quails with average initial weight of 32.09 g of mixed sexes fed diets containing graded levels of sun-dried cassava peel meal were used to study their growth performance. They were randomly allotted to four treatment groups in which cassava peel meal replaced maize at 0, 25, 50 and 75% in a completely randomized design. Each treatment was replicated thrice with feed and water given ad libitum. The parameters measured were feed intake, weight gain, feed conversion ratio and nutrients digestibility, significant ($P < 0.05$) differences were observed in daily feed intake and feed conversion ratio. In the laying phase, 180 female quails were fed with layer's diets with the same levels of replacement and replicated thrice. Significant ($P < 0.05$) differences were observed in the daily feed intake, hen-day production, hen-house production, feed conversion ratio and egg quality traits. Therefore, it was concluded that dietary maize could be replaced with sun-dried cassava peel meal up to 50% for optimum growth performance while 25% level of replacement for optimum egg production in quail diet.

Keywords: Japanese quails, sun-dried cassava peel meal, growth performance and egg production

S1-0191 Effects of body weight development during rearing and reduced crude protein diets in the laying phase on broiler breeder and offspring performance

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A complete investigation from day-old broiler breeder chick to the corresponding offspring grow out was conducted at the Cargill Animal Nutrition innovation center to address 2 research questions: 1) Does growth pattern of breeder pullets during rearing affect breeder and offspring performance and 2) Can reduction of dietary crude protein (CP) during lay control the egg size, without negatively affecting offspring performance. One-day old pullets (n=960) were randomly allotted either to a standard growth pattern or to a pattern aiming at 500 g greater body weight (BW) at start of lay (22 wk of age). Each group consisted of 12 replicates, containing 40 female breeder chicks. At start of lay, 27 females and 3 males were placed per pen. Birds were kept at a 500 g BW difference during the entire laying phase (22-58 wk) by adjusting daily feed intake based on BW. They were fed standard diets until 34 wk of age, thereafter treatments were cross-factored with 2 CP levels (standard 14.4%, vs low 10.7% CP; isocaloric) to study the effects of reduced dietary CP on EP, egg weight (EW), as well as offspring performance. Eggs of hens at 56 wk of age were hatched for the offspring grow out trial, in which growth performance parameters were recorded weekly for 45 days. The higher growth pattern of breeders during rearing resulted in 2.9% greater EP ($P=0.05$) and 2.2% greater EW ($P<0.001$) at the expense of a 14% greater feed intake. Reduced dietary CP did not affect EP, or hatchability. The EW reduced with decreasing dietary CP by 3.8% ($P<0.001$). Decreasing maternal dietary CP levels to 10.5% reduced offspring BW ($P<0.01$), with offspring of heavier hens showing greater BW at that CP level at day 28 than offspring of standard BW hens (1585 vs 1526g; $P<0.06$). Greater breeder pullet BW increases EP and EW with 2.9 and 2.2%, respectively at the expense of 14% greater feed intake, while reduced dietary CP decreased EW with 3.8%.

Keywords: broiler breeder, body weight, rearing program, low protein, egg production, offspring performance

S1-0192 The combination of direct-fed microbials and enzymes improved growth performance in broiler chickens raised with or without ionophores

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Coccidiosis is a major economic burden on the global poultry industry. Increased resistance and loss of sensitivity to traditional anti-coccidial agents has increased the interest in alternative control measures including vaccination, direct-fed microbials (DFM) and gut integrity promoting products. This study aimed to assess the effect of a DFM/multi-enzyme product (DFM + XAP, providing 2000 U/kg xylanase, 200 U/kg amylase, 4000 U/kg protease and 75,000 cfu/g DFM/kg of feed) under two different anti-coccidial strategies (vaccination and traditional ionophore therapy). The study diets were a nutritionally adequate positive control based on corn/soy/DDGS (PC), a negative control reduced by 100 kcal/kg ME (NC), and NC+DFM+XAP. The three diets were divided into two subgroups, receiving either ionophore (salinomycin 60g/t) or a coccidial vaccine (Coccivac B) on day 1 and then fed diets without coccidiostat additions, resulting in 6 treatments (n=8, 50 male birds/n). Growth performance was recorded on days 1, 22, 36 and 43. Effect of DFM+XAP was determined by comparing to birds fed NC and PC diets within the same control program, and impact of coccidiosis control program was analyzed by excluding PC treatments. At day 43 reduced BWG and highest FCR were seen in NC birds, regardless of coccidiosis control ($P<0.05$). Adding DFM+XAP significantly improved BWG and FCR by 5 (2087 vs.1991 g/bird) and 6% (1.803 vs. 1.906), respectively, in birds receiving vaccine ($P<0.05$), resulting in similar BWG (2125 g/bird) and FCR (1.772) to birds fed PC diets. Regardless of control program, adding DFM+XAP resulted in 3% BWG improvement ($P<0.05$). However, even though DFM+XAP improved FCR, it was more effective when birds were given the coccidial vaccine rather than ionophores. In conclusion, the combination of DFM + XAP significantly improved birds BWG, regardless of coccidiosis control program.

Keywords: broiler, direct-fed microbials, carbohydrase, coccidiosis, performance

S1-0193 Impacts of calcium and phytase on inositol phosphates degradation differs depending on segments of digestive tract

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This trial was designed to determine the impacts of calcium (Ca) and phytase on inositol phosphates (IP2-6) degradation in broiler birds. A 2×3 randomized design was used with 2 Ca (0.7 and 1.0%) and 3 phytase (0, 500 and 1000 FTU/kg Butiauxella sp., phytase) levels resulting in a total of 6 treatments (Trt, n=6, 10 birds/n). Broiler birds were raised on floor pens and fed a commercial type starter diet from H to 10 d of age. At 11 d of age, birds were moved to battery cages and the experimental diets (mash) were offered ad lib for two d (11 to 13 d of age). Digesta samples from crop, proventriculus plus gizzard (P+G) and ileum were collected at 13 d of age and analysed for IP2 to IP6 concentrations. Regardless of gastrointestinal tract segments or Ca concentration, IP6 concentration was significantly reduced as a result of phytase inclusion (P<0.05). With 1000 FTU phytase/kg inclusion, IP6 concentration was decreased by 56, 81 and 81% in crop, P+G and ileum, respectively, as compared to birds fed the same diets without phytase inclusion (P<0.05), which was on average, 17 percentage points higher than birds fed 500 FTU phytase/kg diets (P<0.05). As a result of IP6 degradation, increased concentrations of lower IP esters (IP3 to 5) were seen in phytase Trts across all three GIT segments (P<0.05). Phytase did not affect crop or ileal IP2 concentrations, whereas its concentration in P+G was increased by 20% as a result of 1000 FTU phytase/kg inclusion (P<0.05). Ca concentration did not affect either crop or P+G IPs concentrations, except reduced IP4 concentration was seen as a result of increased Ca concentration in P+G (P<0.05). In ileum, increasing Ca from 0.7 to 1.0% increased IP4, 5, and 6 by 31, 50 and 20%, respectively (P<0.05), whereas there were no differences in IP2 and 3 between birds fed the two Ca diets. To conclude, phytase effectively reduced IP6 concentration as early as from crop. The negative impact of Ca on IPs concentration was only seen in ileum.

Keywords: calcium, phytase, inositol phosphates, crop, proventriculus plus gizzard, ileum

S1- 0194 Do dual purpose layers need the same dietary concentration of metabolizable energy as specialized layers?

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Culling 1-day old male layer cockerels is the consequence of the extreme specialization in poultry production towards either egg or meat production. Instead, dual purpose genotypes, with females producing eggs and males fattened for meat, could be used. However, a lower performance and feed efficiency is to be expected with dual purpose genotypes. The aim of the study was to investigate if dual purpose genotypes would better tolerate a lower metabolizable energy (ME) content than a layer genotype and thus would compete less with human food. Ten individually kept hens (laying stage: 28 to 35 weeks) each of 3 dual purpose genotypes (Lohmann Dual, LD; Mechelner, ME; Schweizer Huhn, CH) were compared with a layer hybrid (Lohmann Brown plus, LB). In a cross-over design, each animal received for 4 weeks a control diet (11.5 MJ/kg ME, 39.0 g/kg crude fiber) or a reduced ME diet (10.5 MJ/kg ME, 51.6 g/kg crude fiber) with wheat bran replacing whole wheat. Both diets contained 168 g/kg crude protein. Genotype, diet and the interaction were tested as fixed effects. In addition sequence (fixed) and individual hens (repeated subject) were considered. There was a genotype effect (p<0.001) in most performance and egg quality traits measured but no diet effect and no interaction. Body weights (kg) of the ME were highest (3.4) followed by CH (2.5) and similar in LB (2.0) and LD (1.9). Feed intake (g/d) was higher in ME and LB (114 each) than in CH and LD (103 and 96, respectively). The laying performance was highest for LB (95%), followed by LD (71%) and lowest for ME and CH (54 and 50%, respectively). Feed efficiency (g feed/g egg) was more favorable in LB (1.91) and LD (2.29) than in ME (3.87) and CH (4.56). The order of the average egg weights (g) was LB (63.2), LD (62.0), ME (60.3) and CH (57.5). Egg quality (e.g. concerning Haugh Units) was good (> 74). In conclusion, despite the high performance of LB, all genotypes tolerated a ME content ranging 10% below recommendations.

Keywords: dietary energy, dual purpose poultry, egg quality, laying performance

S1- 0195 Detecting taste thresholds in chicken: a two- alternative forced choice method

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Studying the mechanisms of taste sensing in chicken can increase current knowledge regarding the biological factors that influence their feed intake. The method for examining chicken taste sensing abilities is by conducting a two alternative forced choice (2- AFC) taste test, in which consumption levels of a certain tastant (taste molecule) and a tasteless control are compared. 2-AFC tests were conducted on 3-7 day old broiler chicks to assess their detection thresholds for the bitter, sweet and umami (glutamate) tastes. These tests were held in cages containing three chicks and two water bottles in each cage. One bottle contained a tastant solution and the other contained non- flavored water. In the control group, both bottles contained non- flavored water. Consumption levels from all bottles were calculated after 24 hours. The taste threshold for each tastant was determined the minimal concentration that created a significant difference in consumption compared to the control group. Results showed that broiler chicks sense the bitter tastants quinine, caffeine, erythromycin and nicotine with thresholds of 0.3mM, 10mM, 0.1mM and 0.33mM, respectively, resulting in a significant decrease in tastant consumption. In humans, detection thresholds for these molecules are lower, thus chicken are less sensitive to bitter tastants than humans. The detection threshold for the umami tastant monosodium glutamate is 0.3M, the same as in humans. The chicks' response to this tastant was a reduction in consumption as well. No detection ability was found for the sweet tastant sucrose. In contrast, humans are able to detect this tastant at 0.015M. In this study, a method for detecting taste thresholds for certain tastants and their effects on consumption in broiler chicks was developed. This method could benefit the poultry industry by indicating which feeding ingredients or flavors could enhance or reduce food consumption, due to their taste perception in chicken.

Keywords: chicken, taste, threshold, 2-AFC taste test

S1-0196 Tastant sensing in the broiler gastrointestinal tract

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Taste is crucial for nutritional evaluation, food acceptance and toxin avoidance and affects feed intake and weight gain in mammals. Taste components are expressed in the gastrointestinal tract (GIT) of mammals and are involved in GIT processes such as motility, digestion etc. however, knowledge on tastant sensing in the chicken GIT is limited. The chicken has only 3 bitter taste receptors compared to ~30 in mammals and lack one dimer of the heterodimeric sweet taste-receptor. Our aim was to identify chicken in-vivo detection of different tastants, detect taste-related genes expression in its GIT and the effect of bitter and umami tastant on their expression. Accordingly, we confirmed in-vivo detection for umami and bitter tastants using a 2-choice test method and used qPCR for detection of taste related genes expression in the broiler GIT and analysis of expression changes following bitter and umami tastant administration. The results show expression of bitter taste receptor genes (T2R1, T2R2, T2R7) umami (T1R1 and T1R3) and their downstream protein genes (TRPM5, α - gustducin and PLC β 2) in the upper (palate, tongue and stomach) and lower GIT (small intestine, cecum and colon) of embryo and mature chicken. Administration of a bitter molecule (quinine) decreased expression of 2 bitter taste receptors and brush border enzymes (PepT1 and SGLT-1) in the intestine. Other bitter molecules like erythromycin, nicotine and caffeine affected bitter taste gene expression in the GIT as well. The umami tastant monosodium glutamate changed the umami receptor gene expression in the GIT. Our results of tastants in-vivo detection, taste gene expression and their expression alterations imply on the involvement of taste pathways for sensing amino acids and bitter compounds in the chicken GIT , although the exact mechanism is yet to be revealed. These suggest the future possibility of affecting feeding behavior and GIT processes via specific tastant sensing mechanisms in the broiler GIT.

Keywords: taste, broiler, thresholds, gene expression, GIT

S1-0197 Effect of diet form and xylanase on growth performance and ileal nutrients digestibility of heat-stressed broilers fed wheat-soybean diet

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The objective of this experiment was to evaluate the effects of xylanase and feed form on the growth performance, ileal nutrient digestibility, and jejunal morphology of heat challenged-broilers fed wheat-soybean based diets. The experiment was conducted as a 3×2 factorial arrangement with three feed forms (mashes, crumbles, and pellets) and two levels of xylanase (without or with 300 mg/kg of the diet). Each of the 6 dietary treatments were fed to 5 replicate pens (8 birds/pen) from 0 to 42 d of age. Broilers fed mash had lower average daily feed intake, average daily gain, ileal digestibility values of apparent metabolizable energy corrected to zero nitrogen retention (AMEn) and crude protein (CP), villus length and villus length: crypt depth ratio in the jejunum segment than broilers fed crumbles or pellets. The crypt depth in the jejunum was lower ($P<0.001$) for broilers fed crumbles or pellets than for broilers fed mash. Xylanase significantly increased ADG and ileal digestibility of AMEn, CP, and Ca. Overall, the results indicated that either crumbling or pelleting of the diets improved growth performance and ileal nutrients digestibility of broilers subjected to heat challenge. It could be concluded that feeding crumbled and pelleted diets may offer a suitable nutritional strategy to improvement productive performance of broilers reared under high ambient temperatures.

Keywords: broilers, feed form, heat stress, wheat, xylanase enzyme

S1-0198 Effect of polysavone on laying performance and feed nutrient utilization of laying hens at peak production

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The study was conducted to evaluate the effects of dietary supplementing with polysavone on performance, egg quality and feed nutrient utilization of laying hens at peak production. A total of 540 26-week-old Nick laying hens were assigned into 5 groups consisting of 6 replicates of 18 birds each pen. The layers in control group were fed with corn-soybean basal diets, other groups were fed with basal diets supplemented with 300, 600, 900, 1200 mg/kg polysavone. The experimental contains pre-experiment (7 days) and of experiment period (70 days). The results indicated the laying rate of 900 mg/kg and 1200 mg/kg polysavone significantly increased by 2.65% and 2.27% ($P<0.05$) compared with control group. The group of 900 mg/kg polysavone significantly increased average egg weight by 2.14% compared with control group ($P<0.05$). Dietary polysavone did not affect the feed/egg ratio ($P>0.05$). Eggshell strength and haugh unit of 600 mg/kg polysavone were significantly increased by 5.10% and 2.28% compared with control group ($P<0.05$). The group of 900 mg/kg polysavone affected egg weight, eggshell strength, albumen height and haugh unit, which increased by 2.37%, 3.59%, 4.59% and 1.80%, respectively, compared with control group ($P<0.05$). No differences in the egg shape index, shell thickness, yolk color and the ratio of egg yolk to albumen were observed among the experimental groups ($P>0.05$). The group of 900 mg/kg polysavone was significantly higher on apparent metabolic rate of energy, CP and Ca, which increased by 7.82%, 9.30% and 9.01%, respectively ($P<0.05$) compared with control group, while no difference was found between five groups on apparent metabolic rate of total phosphorus ($P>0.05$). Dietary polysavone did not affect liver, spleen, heart relative index, however, the abdominal fat content significantly decreased ($P<0.05$). Therefore, polysavone can improve laying performance, egg quality and nutrient utilization of laying hens, the optimum level is 900 mg/kg.

Keywords: polysavone, laying hen, laying performance, egg quality, nutrient utilization

S1-0199 Effects of diet form and xylanase on biomarker indicators and bone mineralization of heat-stressed broilers fed wheat-soybean diet

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A total of 240 broilers were used to study the effects of xylanase supplementation and feed form on the bone mineralization and tibia breaking strength, and heat stress biomarkers in heat-stressed broilers fed wheat-soybean based diets. From 0 to 42 d of age, there were 6 treatments with factorial arrangement of three feed forms (mash, crumbles, and pellets) and two levels of xylanase (without or with 300 mg/kg of diet). Broilers fed crumbles or pellets had higher tibia breaking strength, than broilers fed mash. The tibia ash, Ca and P contents in ash were greater ($P<0.001$) for broilers fed pellets than for broilers fed either mashes or crumbles. The circulating heterophil-to-lymphocyte ratio, creatine kinase level and heat shock protein 70 mRNA of breast muscle were also decreased ($P<0.05$) by both the crumbles and pellets diets. Xylanase significantly increased tibia ash, tibia Ca and P contents ($P<0.05$). The results indicated that crumbling or pelleting of the diets improved minerals retention and down-regulated heat stress biomarkers of broilers subjected to heat challenge. It could be concluded that feeding crumbled and pelleted diets may effectively partially ameliorating the resistance to heat-stressed birds.

Keywords: broilers, heat stress, biomarkers, feed form, wheat, xylanase

S1-0200 Roxazyme G2G supplemental value in the utilization of cassava starch residue by broiler-chickens

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The study assessed the supplemental value of roxazyme G2G in the utilization of cassava starch residue (CSR) by broiler-chickens using performance and cost implications as the response criteria in an eight week feeding trial involving 450 day-old Marshall breed of broiler-chicks. Fifty broiler-chicks were randomly distributed to each of the nine dietary treatments, replicated five times of ten birds per replicate. The experimental design was the completely randomization with 3 x 3 factorial arrangements of treatments. Cassava starch residue was used to replace maize in the diets at 0, 20 and 40% levels and enzyme supplementation was at 0, 100 and 200 mg/kg to each level of CSR inclusion. Data were analyzed using SAS and the means separated using Duncan new Multiple Range Test. Results showed that birds fed diet containing 20% CSR as substitute for maize were similar ($P>0.05$) in the two physiological growth phases (starter and finisher) to the birds fed the control with respect to average daily weight gain (24.02 versus control: 24.24 g/b/d, and 40.94 versus control: 40.82 g/b/d, respectively) while enzyme supplementation at 100 mg/kg numerically enhanced the replacement of maize with CSR up to 40% at both phases without compromising the weight gain of the birds. However, CSR x Enzyme supplementation interaction was not significant ($P>0.05$) suggesting that the trial could be independent of the two factors with respect to weight gain of the birds. Cost of feed \$/kg weight gain significantly ($P<0.05$) decreased in broiler-chickens fed on CSR meal with 100 mg/kg enzyme supplementation with significant ($P<0.05$) interaction of CSR x Enzyme supplementation implying the dependency of cost of feed \$/kg weight gain on these factors. The study revealed that replacement level of CSR for maize in broiler-chicken diet could be increased to 40% with roxazyme G2G supplementation at 100 mg/kg.

Keywords: broiler, cassava waste, roxazyme G2G

S1- 0201 Effect of dietary butyrate release location on gut functions and protein digestibility of broilers in a feed challenge model

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An experiment was conducted to investigate the effect of providing butyrate (Bt) to distinct gastrointestinal tract (GIT) segments on protein digestibility of broilers. It was hypothesised that providing Bt to distinct GIT segments would lead to differential effects on digesta retention time, gut morphology and proteolytic enzymatic activities, ultimately resulting in differences in protein digestibility. A total of 320 male day-old Ross 308 broilers were randomly assigned to 5 dietary treatment groups: 1) Control (no butyrate), 2) unprotected Bt (activity in the crop and gastric regions), 3) tributyrin (activity in the small intestine), 4) fat-coated Bt (activity in whole GIT) and 5) unprotected Bt combined with tributyrin, each replicated 8 times. Bt was included at 1 g/kg, as fed basis. Rapeseed meal was used in combination with a fine dietary particle size in order to challenge the digestive capacity of young broilers. Birds were dissected at 22 d of age. Butyrate concentration varied significantly across GIT segments, indicating that dietary contrasts in Bt release location were successful. Irrespective of its release location, Bt tended to reduce feed intake. Butyrate delivery to the crop and gastric regions increased significantly intestinal retention time and proteolytic activity of the proventriculus. Butyrate delivery to the colon/ceca increased significantly intestinal retention time and relative weight of the jejunum, resulting in an enhanced feed efficiency tendency. Intestinal morphology and apparent ileal digestibility of nitrogen were unaffected by dietary treatments. Overall, these results indicate that digestive functions are affected by dietary Bt supplementation and that the GIT segment wherein Bt is released modulates these changes. However, such changes did not accumulate into an improvement of nitrogen digestibility, indicating that effects on microbiota and immune competence could explain the positive effects of Bt supplementation.

Keywords: butyrate, release location, protein digestibility, passage rate

S1-0202 In-ovo inoculation of raffinose (as a prebiotic) improves hatchability rate and gut health, but not growth performance of broiler chickens

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The effects of in-ovo inoculation of raffinose (RFO) as a prebiotic on hatchability rate, growth performance, relative weight of proventriculus, gizzard, drumstick and breast muscles, ileum mucosa morphology and immune related gene expression were examined in Cobb 500 broilers. A total of 168 fertilized eggs were divided into four groups: a control with no inoculation and 3 levels of RFO solution (1.5, 3.0, and 4.5 mg in 0.2 ml of a commercial diluent) inoculation. The RFO solution was injected into air sac on d 12 of incubation. Hatched chicks were grown on a standard diet and management, and feed intake and body weight were recorded weekly. The birds were sacrificed on d 21 post hatch for collection of samples. Total RNA was extracted from small intestine, and RT-qPCR was performed to evaluate immune response by quantifying mRNA levels of marker genes of immune cells. Inoculation of RFO had no significant effect ($P>0.1$) on d 1 body weight of chicks, but hatchability rate increased linearly ($P<0.05$) with increasing dose of RFO (72.1, 76.3, 78.3, and 82.8%, respectively). On d 21, the relative weight of proventriculus, drumstick and breast muscles were not affected ($P>0.1$) by RFO, but relative weight of gizzard increased linearly ($P<0.05$). On hatch day, villus height increased linearly ($P<0.01$) with increasing dose of RFO. Also, increasing dose of RFO increased the villus height and villus height:crypt depth ratio ($P<0.05$) but did not affect the crypt depth on d 21. The expression levels of CD3 (a T-cell marker) and $\chi B6$ (a B-cell maker) were significantly enhanced by increasing dose of RFO. In conclusion, although in-ovo inoculation of RFO did not significantly influence growth performance or slaughter yield of broilers, RFO has the potential of increasing hatchability rate and improving gut health via vitalizing ileum mucosa morphology and immune response.

Keywords: broiler chicken, gene expression, growth performance, ileum mucosa morphology, in-ovo injection, prebiotic

S1-0203 Role of natural growth promoters (AV/AGP/10 and Vilocym Z) as antibiotics alternatives in broilers

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The objective of study was to evaluate the effects of natural growth promoters of AV/AGP/10 and Vilocym Z on performance, carcass characteristics, intestinal morphology, nutrient utilization, immunity and liver function in broilers. A total of 600, 1-day-old Cobb 500TM broilers were assigned to 1 of 4 treatments with 6 replicates of 25 birds each using a completely randomized design and were fed a diet containing 0.05% sand (NC), 0.05% BMD 50 (PC), 0.05% AV/AGP/10 or 0.05% Vilocym Z for 42 d. The feed intake and body weight were recorded at 2, 4 and 6 wks. Six birds per replicate were selected to measure the carcass characteristics at the end of study. At 14d and 42d, small intestine was collected from 1 bird per replicate for morphology analysis. At 36d, litter ammonia emission were measured in each pen. At 42d, ileal digesta was obtained from 4 birds per replicate to measure digestibility; blood were collected from 1 bird per replicate for analysis of blood metabolites and WBC differentiation; liver and ileum for mRNA expressions. Performance were unaffected by treatments ($P>0.05$), except for the lower FCR in PC than NC at 3 to 4 wks ($P<0.1$). Broilers fed the AV/AGP/10 diet had higher percentage of wing ($P<0.05$) and leg ($P<0.1$) compared with other treatments and PC, respectively, and greater jejunum villus height/crypt depth ratio at 14d compared with others ($P<0.05$). Compared with NC, the digestibility of protein and AMEn were increased and ammonia emission was decreased in other three treatment ($P<0.05$). The levels of liver function markers (AST, etc.) were all in normal levels and no difference of H/L ratio, levels of serum IgG and gene expression of IL-6, IL-10, INF- γ were found among treatments ($P>0.05$). These findings suggest that AV/AGP/10 and Vilocym Z can be potential antibiotics alternatives in terms of improved meat characteristics, nutrient utilization and decreased ammonia emission as well as no negative effects on liver and immune function.

Keywords: AV/AGP/10, Vilocym Z, performance, nutrient digestibility, ammonia emission

S1-0204 Supplemental enzymes benefit necrotic enteritis challenged broilers

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The present study investigated whether reducing viscosity in gut environment, breaking down of soluble and insoluble NSP using dietary supplementation of xylanases benefit necrotic enteritis challenged broilers. A 2×3 factorial arrangement, with or without necrotic enteritis (NE) challenge and three enzyme supplements, was applied to 468 Ross 308 male day-old broilers in floor pens. Dietary enzyme supplementations (DSM WX, DSM VP and DSM ProAct) were formulated into basal diets comprises mainly wheat, soybean, meat meal with recommended nutrient compositions but a lower ME, e.g. starter 2950 kcal, grower 3000 kcal, finisher 3050 kcal. Necrotic enteritis (NE) challenge was performed by single dose per os of 1 ml *Eimeria* species (*E. acervulina*, 5000 oocytes; *E. maxima*, 5000 oocytes; *E. brunetti*, 2500 oocytes) at d 9, and followed by *C. perfringens* type A strain EHE-NE18 (CSIRO Animal, AU) broth (approx. 108 CFU) per os at d 14 and d 15. The study applied appropriate managements and the cumulative pen performance was recorded on d 12, d 16, d 20, d 24 and d 35. A significant depression ($p<0.01$) of feed conversion ratios (FCR) was evident in the necrotic enteritis challenge groups from d 16 up to d 35. The enzyme supplements showed a beneficial effect ($p<0.05$) in FCR among WX and VP supplemented treatments (WX: 1.372; VP: 1.383) at d 35, compared with non-xylanase supplemented treatments (ProAct: 1.416). Although, a full factorial interactions did not suggest significances across treatments compelled with the necrotic enteritis challenge, but a profound improvement ($p<0.05$) on FCR was seen by DSM WX with challenge at d 12. In conclusion, dietary supplementation of DSM WX and DSM VP can provide some level of improvements on FCR against necrotic enteritis in broilers.

Keywords: supplemental enzyme, necrotic enteritis, challenge, FCR

S1- 0205 The application of dietary electrolyte balance in broiler nutrition under fully plant origin low protein diet supplemented with exogenous protease

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Animal origin protein is getting less and less so plant origin protein becomes the main source of animal nutrition. The exogenous protease has played a major part in helping to release plant origin protein nitrogen, which are trapped within insoluble fibers cell walls, and radically improve the efficiency of meat production. The beneficial effects documented are beyond doubt, but the responses obtained are variable and sometimes lacking. Therefore the study was to investigate the effects of dietary electrolyte balance (dEB) value in fully plant origin low protein diets supplemented with exogenous protease on the growth performance of broiler under high ambient temperatures. 800 Arbor Acres broiler were randomly allocated to five 5 feeding treatments of four replicates. There were 40 birds kept in a 3.8m² floor feeding pen of which ambient temperature was between 28~32°C. In 1-3 weeks feeds contained 21% CP, and 19% for 4-5 weeks, energy level are the same for the two stages (3200 Kcal/kg feed). Treatment 1: control group (calculated 225 meq Na⁺, K⁺, Cl⁻/kg), Treatment 2: adding water electrolyte (calculated 50 meq/L, water: feed ratio=2 L/kg), Treatment 3: dietary exogenous protease (1,000 U/kg feed), Treatment 4: dietary electrolyte (calculated 325 meq/kg), Treatment 5: dietary exogenous protease and electrolyte. The results showed that the 5th week body weight (P=0.004), overall body weight gain (P=0.004) and feed intake (P<0.001) were increased significantly between treatments. By using protease to improve dietary protein digestibility or increase dEB value could improve growth performance, it also has the results of addition and multiplication of these two methods.

Keywords: dietary electrolyte balance, protease, high ambient temperature, broiler

S1- 0206 The application of dietary electrolyte balance in broiler nutrition under fully plant origin low energy level diet supplemented with xylanase

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The exogenous xylanase is widely assumed to have the ability to degrade plant cell and release nutrients from endosperm and aleuronic layer cells. The beneficial effects of xylanase in increasing energy density for diet are documented beyond doubt; the responses obtained are variable and sometimes lacking. Therefore the study was to investigate the effects of dietary electrolyte balance (dEB) value in fully plant origin low energy level diets supplemented with exogenous xylanase on the growth performance of broiler under high ambient temperatures. 800 Arbor Acres broiler were randomly allocated to 4 feeding treatments of five replicates. There were 40 birds kept in a 3.8 m² floor feeding pen of which ambient temperature was between 32~28°C. In 1-3 weeks feeds containing 23% CP, 4-5 weeks with 21% CP, two stages energy level are the same as 3100 Kcal/kg feed. Treatment 1: control group, Treatment 2: dietary xylanase (16,000 U/kg feed) and water electrolyte (calculated 50 meq/L, water: feed ratio=2 L/kg), Treatment 3: dietary exogenous xylanase, Treatment 4: dietary xylanase (16,000 U/kg feed) and electrolyte (calculated 325 meq/kg feed). The results showed that the 5th week body weight (P=0.0404) and overall body weight gain (P=0.0307) were significantly increased between treatments. By using exogenous xylanase to increase dietary energy level or increase dEB value could improve growth performance, it also has the results of addition and multiplication of these two methods.

Keywords: dietary electrolyte balance, xylanase, high ambient temperature, broiler

S1- 0207 Bacterial community in broiler intestinal caeca in connection with nutritive diets of different composition

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The objective of this study was to analyze the bacterial community in broiler caecum under diets of various composition using molecular-genetic methods. The experiments were conducted on broiler chickens of Cobb 500 cross in the vivarium of «Zagorsky Experimental Breeding Farm». 21-day old chickens were arranged in 4 groups, each of 35 specimens, and fed with diets based on 60% corn or 45% barley or 25% sunflower meal or 60% wheat. Live weight was measured in broilers at days 21 and 37. By the end of the experiment, microflora of 3 birds for each group was analyzed by NGS and real-time PCR methods. Typical inhabitants of the avian gastrointestinal tract were found – families Clostridiaceae, Eubacteriaceae, Lactobacillaceae, phylum Bacteroidetes, as well as new unidentified taxa and bacteria of families Lachnospiraceae and Ruminococcaceae, previously considered as characteristic ruminal bacteria. Enterococci and bifidobacteria were minor. The composition of a diet affected significantly the caecal microbiome. The highest average daily gain in the avian live weight was registered under fiber-deficient feeds (60% corn and 25% sunflower meal). Here, the total number of bacteria was decreased 10-fold, against an increased proportion of phylum Bacteroidetes and family Clostridiaceae, possessing enzymes for digesting starch polysaccharides. Increased the amount of bacteria of genus *Escherichia* and family Sutterellaceae that can cause intestinal dysbiosis were observed. Thus, detailed changes in the composition of previously known and new unidentified bacteria in broiler caecum under the diets of different composition were revealed by using molecular genetic methods. The link between the intestinal microflora and broiler productivity was established. The study was supported by the Russian Science Foundation (project no. 141600140 «Modern Views on the Intestinal Microflora of Poultry with Different Diets: a Molecular Genetic Approach»)

Keywords: intestinal caeca, bacterial community, NGS, real-time PCR

S1-0208 Garlic improves absorptive surface of intestine in pulmonary hypertensive chickens

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Garlic (*Allium sativum*) as a medicinal plant has been used in the prevention and treatment of many intestinal disorders and metabolic diseases. The aim of this study was to investigate the effects of dietary garlic on the intestinal absorptive surface in broiler chickens with 3,5,3'-l triiodothyronine (T3)-induced pulmonary hypertension. One hundred and eighty, one-day-old fast-growing broiler chickens (Ross 308) were assigned to 4 groups (one control and three treatments) with 3 replicate pens per group and 15 chickens per pen and reared for 42 days. T3 was added (1.5 mg/kg) in the grower and finisher diets. For the treatments, garlic powder was included in the standard basal diet at a concentration of 0.2, 0.6 or 1% of the diet. At 14, 28 and 42 days of age, 9 chicks randomly were selected from each group at each age, decapitated and their morphometric variables of intestine (villus length and width) measured and then villus surface area was calculated (villus surface area = length × width × π) in the duodenum, jejunum and ileum. At 42 days, the heart was dissected and then the right ventricle to total ventricle ratio (RV:TV) was calculated. Chickens with a RV:TV \geq 0.29 were classified as pulmonary hypertensive chickens. Comparisons were made by using one way ANOVA. Chickens with RV:TV \geq 0.29 were decreased in all garlic-groups compared to control. The duodenal surface area was greater in chickens fed garlic supplement (0.2, 0.6 and 1%, day 42; 0.6 and 1%, day 28) than control. The jejunal villus surface area was higher in chickens fed garlic supplement (all concentrations, day 28; 0.6 and 1%, day 42) than controls. The ileal villus surface area in 0.6% garlic group was also higher in chickens fed garlic supplement than control at day 42 ($P < 0.05$). Garlic supplement not only modulates pulmonary hypertensive response but also improves absorptive surface area of intestine.

Keywords: ascites, broiler chicken, garlic, intestinal villi, pulmonary hypertension

S1-0209 Effects of propolis, royal jelly, bee pollen and Ronozyme supplementation in diets of Japanese quails (*Coturnix coturnix japonica*) on yolk lipid peroxidation

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This experiment was conducted to investigate the effects of propolis, royal jelly, bee pollen and Ronozyme supplementation in diets of Japanese quails (*Coturnix coturnix japonica*) on contents of malondialdehyde (MDA) in yolk. Total one hundred and sixty Japanese quails at 43 days of age were used and divided into 4 replicate groups each containing 32 animals. The experimental groups as follows: Group 1 (control) was feed a basal diet, group 2 (propolis) was feed a basal diet supplemented with 4 g/kg diet, group 3 (royal jelly) was feed a basal diet supplemented with 500 mg/kg diet, group 4 (bee pollen) was feed a basal diet supplemented with 1 g/kg diet, group 5 (Ronozyme) was feed a basal diet supplemented with 1 g/kg diet, orally. The experiment was continued during 74 days. All eggs were collected on end of experiment and the yolk were evaluated MDA levels. MDA levels of the yolk were found highest in the control, royal jelly and Ronozyme groups as compared with bee pollen and propolis groups ($P<0.05$). MDA levels of the yolk in bee pollen and propolis groups were significantly lower than those of other groups ($P<0.05$). MDA levels of yolk in bee pollen and propolis groups were found to be similar. MDA levels was significantly improved in the bee pollen and propolis groups as compared with royal jelly and Ronozyme groups ($P<0.05$). The results of the current study showed that bee pollen and propolis supplementation to quails diet has improved much more than those of royal jelly and Ronozyme supplementation to lipid peroxidation. Inclusion of bee pollen and/or propolis to diet decreased lipid oxidation and prolonged the shelf life of egg, in case of bee pollen and propolis having unsaturated fatty acid composition consumed by layers. In conclusion, bee pollen and propolis used as alternative to antioxidants had possitive effects on lipid peroxidation. They can be used as preventing lipid oxidation.

Keywords: Japanese quails, bee pollen, propolis, royal jelly, Ronozyme, lipid peoxidation

S1-0210 Effect of Chinese medicinal herbs on SOD and GSH-Px activities and mRNA expression in the kidney and liver tissue of laying hens

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The experiment were conducted to identify the impact of Chinese herbal compound prescription (Glycyrrhiza, Forsythia suspense, Lonicera japonica, Oregano, Astragalus, Ginkgo Biloba leaves, Dry Zingiber) on activities and expressions of mRNA and GSH-Px in kidney tissue and liver tissue in layers. 3072 layers were randomly allotted to 4 groups with 4 replicates with 192 chickens in each replication and fed for 30 days. Experimental treatments including 0g/t, Chinese medicinal herbs (CMH) were used. The results showed that Superoxide dismutase (SOD) activities in kidney tissues were significantly increased as well as SOD mRNA expression when diets was supplemented with 50 g/t, 100g/t, or 150g/t CMH ($P<0.05$); Glutathione peroxidase activities (GSH-Px) were dramatically increased when diets was supplemented with 50 g/t and 100g/t CMH respectively ($P<0.05$); CMH 50 g/t was added in diets remarkably increased GSH-Px mRNA expression ($P<0.05$). SOD activities in the liver had a tendency to increase when diets was supplemented with 50 g/t and 100g/t CMH respectively ($P>0.05$); and GSH-Px activities in treatment groups were improved ($P>0.05$); Dietary CMH supplement at 100g/t increased SOD mRNA expression ($P>0.05$), and CMH Addition at 150g/t improved GSH-Px mRNA expression ($P>0.05$). It indicated that CMH increased the activities of SOD and GSH-Px by regulating the expression of SOD mRNA and GSH-Px mRNA. In conclusion, the results from the current study suggest that feeding CMH may have a beneficial effect on antioxidant capacity in the kidney and the liver of laying hens as well as provides a useful insight to the development of potentially useful new feed additive supplements.

Keywords: laying hens, glutathione peroxidase, superoxide dismutase, mRNA expression

S1-0211 Effect of phytase dose and Ca level on performance in broilers fed corn soybean meal based diets with reduced nutrient density

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This study tested the effect of phytase dose and Ca level on performance of broilers fed a corn/SBM based diet. Nine treatments were tested including a positive control (PC, based on breeder's recommendations). A basal diet was formulated without Ca and P supplements and with reduction of 68 kcal ME/kg, on average 0.02% dig AA and 0.03% Na vs PC. This basal diet was supplemented with *Buttiauxella* sp. phytase, DCP and limestone: 3 diets at 500 FTU/kg with reduction of 0.146% AvP, 0.134, 0.164, 0.234% Ca respectively (low, medium, high) vs PC; 3 diets at 1000 FTU/kg with reduction of 0.174% AvP and 0.159, 0.189, 0.234% Ca; 2 diets at 2000 FTU/kg with 0.183% AvP, 0.196 and 0.234% Ca reduction (medium and high). Filler was used to maintain the same ingredient composition in all phytase treatments except DCP and limestone. Ross 308 male broilers were used with 8 replicates (20 birds/pen) in a randomised block design. Pelleted diets were offered ad libitum for 0-42 days. At 42d, tibia samples (5 birds/pen) were collected. No Ca level and phytase dose interaction was found, the main effects of phytase dose and Ca level were tested by Tukey's HSD using JMP 11 (SAS). All phytase treatments compensated nutrient reduction, with BWG and FCR equal to or better than PC (superior to the breeder targets). Phytase at 2000FTU/kg improved BWG (0-21d), reduced FCR and feed cost/kg BWG (0-10d) vs 500FTU/kg, while 1000FTU/kg showed lower FCR (0-21d). Linear or curve-linear responses were observed with increasing phytase dose on BWG and FCR. Reducing Ca level lowered feed cost/kg BWG and improved energy efficiency in a step wise manner, the high Ca reduction showed lower feed cost/kg BWG and higher energy efficiency ($P < 0.05$) than low Ca reduction, suggesting a better Ca/P balance. Bone ash was not affected. The data suggest that high phytase dose (1000- 2000FTU/kg) may lead to production benefit, while the impact of Ca level need to be further evaluated under different dietary conditions.

Keywords: broilers, *buttiauxella* phytase, calcium, available p, production performance

S1- 0212 Development of calibrations for evaluation of calcium level in limestone by using near infrared reflectance spectroscopy

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Near infrared reflectance spectroscopy (NIRs) is an important tool for rapid analysis and prediction of feed ingredients. Calcium is very important for both in broiler and laying chicken's diet and limestone is the best source for that. In Bangladesh, we collect limestone mainly from Bhutan and sometimes from Vietnam. Near infrared reflectance spectra were collected from a FOSS 5000 Reflectance Spectrophotometer using a Quartz Quarter cup sampling device. Multivariate analysis was performed for the development of calibration equations of nutrients by WinISI 4.6 software to relate the spectral data and corresponding concentration values of limestone. Around 1000 sample data were centered using the partial least squares algorithm, and spectral outliers were identified from each calibration. The accuracy of the calibration models was validated by root mean square error cross validation (RMSECV), root mean square error of estimation and correlation coefficient (R^2) between the measured values of nutrient component determined by analytical laboratory vs. predicted values. Equation was selected on the basis of lowest Standard Error of Cross Validation (SECV) and highest 1 minus the Variance (1-VR) for calcium (Ca) content. It is concluded that NIRs could potentially be used to predict Ca content in Limestone.

Keywords: NIR, calibration, poultry, limestone

S1-0213 Effects of dietary L- arginine levels on the intestinal protein turnover and the expression of genes related to protein synthesis and proteolysis of laying hens

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The present study was conducted to test the effects of dietary L-arginine (Arg) levels on protein metabolism on the intestinal of laying hens and the expression of genes related to protein synthesis and proteolysis. Xinyang black commercial laying hens (n = 864, 31 wk of age) were randomly allocated to 6 treatments with 4 replicates of 36 birds. The dietary treatments were corn- corn gluten meal based diets containing 0.64, 0.86, 1.03, 1.27, 1.42, and 1.66% L-Arg, respectively. Results showed that, the fractional protein synthesis rate (FSR) and fractional protein gain rate (FGR) in the jejunum increased firstly and then decreased along with the increasing L-Arg, and the 1.27% L-Arg group had the highest FSR and FGR. Consistent with the data on protein turnover, mRNA abundances of ribosomal protein S6 kinase 1 (S6K1) in the jejunum of layers fed 1.27% L- Arg was the highest, which was 2.70-fold higher ($P<0.05$) than that in the 0.64% L- Arg group. Similarly, mRNA expression of target of rapamycin (TOR) in the jejunum was maximized in the 1.27% L-Arg groups. In the duodenum, the mRNA abundances of TOR and S6K1 was maximized in the 1.27% L-Arg groups, 2.03- fold ($P<0.05$) and 2.58- fold higher ($P<0.05$), respectively, than that in the control group (0.64% L-Arg). While both TOR and S6K1 were not significantly influenced by dietary treatments in the ileum. The mRNA abundances of cathepsin B in the ileum, as well as the 20S proteasome in the duodenum and the jejunum, decreased at the same dietary L-Arg level of 1.27%. In conclusion, the dietary level of L-Arg mainly affects the protein turnover in the jejunum of laying hens, and the action of an appropriate level of dietary L-Arg involves upregulating the gene expression of the TOR signaling pathway in the intestinal.

Keywords: laying hens, L- arginine, gene expression, intestinal, protein turnover

S1-0215 The effect of a carbohydrase complex on performance and energy utilization of corn- SBM- fed broilers with different dietary energy and amino acid levels

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A new carbohydrase (Rovabio® Advance) has been developed to enrich both total amount and the diversity of protein enzymes of xylanase and arabinofuranosidase activities. The present study was carried out to evaluate the effect of this enzyme complex on the performance and energy utilization of broilers chickens fed corn and soybean meal-based diets with reduced levels of metabolizable energy (ME) and digestible amino acids (dAA), between d1- 42. Two thousand and sixteen day-old male Cobb 500 chicks were randomly distributed into nine treatments, with eight replicates of 28 chickens in each. The treatments were Positive Control (PC); Negative Control 1 (NC1, PC - 80 kcal ME/kg & -3% dAA); NC2 (PC -80 kcal ME/kg & - 6% dAA); NC3 (PC - 120 kcal ME/kg & - 3% dAA); and NC4 (PC - 120 kcal ME/kg & - 6% dAA). Those four NC diets were either supplemented with the new carbohydrase complex, or they were not. The positive control diet was formulated based on Brazilian Tables, and all feeds were provided in mash form. At d21 and d42, four birds per experimental unit were randomly selected for ileal digesta collection and determination of metabolizable energy (ME). In terms of BWG, birds were not sensitive to the reduction of ME and dAA content; however, the FCR was significantly degraded when birds were fed with the NC2, NC3, and NC4 diets compared to PC. The addition of the carbohydrase complex to the NC diets systematically improved FCR to the level of the PC. The average improvement of FCR obtained by the addition of the enzyme was 2.9%. Based on ileal digesta analysis, the addition of the carbohydrase complex improved ME by 4.3% on average at d21 and by 2.6% at d42. The addition of the evaluated enriched xylanase and arabinofuranosidase enzyme complex enhanced utilization of feeds' energy, allowing recovery of performance losses promoted by reduced dietary levels of ME (up to - 120 kcal/kg) and dAA (as far as -6%) in corn-SBM fed broilers chickens up to 42 days of age.

Keywords: arabinofuranosidase, xylanase, performance, metabolizable energy, amino acids

S1- 0216 Antimicrobial action of chestnut tannin and monoglycerides on in vitro growth of pathogenic bacteria

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Tannins and short chain fatty acids exert a strong antimicrobial action upon pathogenic bacteria and may be used as substitutes for antibiotics in feeds. Objective of the experiment was to confirm the antimicrobial efficacy of a chestnut hydrolysable tannin (CT) in the liquid form and of a blend of 1-monoglycerides of saturated short chain fatty acids (SA), from butyric (C4:0) to capric (C10:0), and to check the possible synergic effect of their mixtures, by means of an in vitro experiment. Bacterial strains were: *Clostridium perfringens*, from birds with necrotic enteritis; *Salmonella typhimurium*, from layers; *Escherichia coli*, from birds with colibacillosis, *Campylobacter jejuni*, from the skin of broilers. Each bacterial sample was diluted in BHI broth to the concentration of 2×10^3 cfu/mL and 5 mL of each suspension were then mixed with 5 mL of the product to be tested: 0.6% CT, the T group; 0.6% SA, the M group; 0.4% CT and 0.2% SA, the TM group; 0.2% CT and 0.4% SA, the MT group. Triplicates were incubated at 37°C (44°C for *Campylobacter*). Viable bacterial counts were performed after 30 minutes, 3 hours and 24 hours. SA resulted more efficient in the short term, whereas CT was more performing in the long term. With *Clostridium* at 24 hours the counts were: 3.5×10^9 the control (C); 200 the T group, 2.3×10^5 the M; 800 the TM and 4,000 the MT. With *Salmonella*: 8×10^7 the C; 3,500 the T, 40,000 the M; 12,000 the TM and 20,000 the MT. With *Escherichia*: 7×10^9 the C; 2×10^5 the T; 8×10^7 the M; 3.5×10^5 the TM and 1.2×10^6 the MT. With *Campylobacter*: 4×10^5 the C; 1,300 the T; 25,000 the M; 10,000 the TM and 16,000 the MT. In conclusion, CT resulted more efficient, both alone and in mixtures with SA. No synergic effect could be detected. Nevertheless a blend appears advisable, due to the contribution of fats to the energy of the diet.

Keywords: chestnut tannin, short chain fatty acids, monoglycerides, antimicrobials

S1-0217 OptiPhos® outperforms Axtra® Phy on speed of phytic acid hydrolysis

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In poultry, phytic acid can only be degraded by a phytase in a pH range below 4.5, being the area of the proventriculus and the gizzard, where phytic acid is in a soluble state. Due to the high transit time in this part of the gastro-intestinal, a phytase should also work at a high speed. The specific aim of this trial was to evaluate the phytate degradation speed of OptiPhos® phytase, using Michaelis-Menten kinetics at this pH range, and to empirically compare it with Axtra® Phy phytase. The pure phytase molecules of OptiPhos® and Axtra® Phy, were purified from commercially available OptiPhos® and Axtra® Phy products. In brief, the phytases were solubilized overnight at 18 °C in 200 ml Tris-HCL buffer followed by a filtration on glass fibre pre-filter of 1 µm, on a membrane filter of 0.45µm and finally on a 0.22 µm GP Express PLUS Membrane. The protein solutions were concentrated by diafiltration. Proteins were precipitated by ammonia sulphate, and the phytase fraction was separated by chromatography. Michaelis-Menten kinetics were determined for the phytases in a Glycine-HCl buffer at pH levels of 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5, after which Vmax and Kcat were calculated. Vmax represents the maximum rate of speed for P release from phytate, at maximum (= saturating) concentrations of the phytate. Kcat is the turnover number, which is the maximum number of substrate (phytate) molecules converted per enzyme molecule per second. Results indicated that the Kcat value and the Vmax of OptiPhos® was higher compared to Axtra® Phy at all pH levels, in particular at lower pH. On average over all pH levels, the Kcat value of OptiPhos® was 893 per sec. vs 529 per sec for Axtra® Phy, while the Vmax value of OptiPhos® was 1045 vs 681 µmol P/min per mg enzyme for Axtra® Phy. It could be concluded that OptiPhos®, compared to Axtra® Phy, works faster in breaking down phytic acid at different pH levels.

Keywords: phytase, Michaelis-Menten, broilers, optiphos, axtra phy

S1-0218 OptiPhos® demonstrates to be a very efficient phytase in turkeys

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A trial was set up to determine the effect of OptiPhos® phytase at different inclusion levels on turkey performance. Six hundred BUT Big-6 male turkeys were divided over 60 pens and fed during 6 weeks wheat/corn/soy based feeds in 4 phases (starter: 2780 kCal ME/kg, 28 % CP, 12 g/kg Ca and 6 g/kg aP; grower I: 2870 kCal ME/kg, 25.5 % CP, 10 g/kg Ca and 5 g/kg aP; grower II: 3020 kCal ME/kg, 22.5 % CP, 8.5 g/kg Ca and 4.3 g/kg aP; finisher: 3160 kCal ME/kg, 19 % CP, 7.5 g/kg Ca and 3.8 g/kg aP). Six treatments were imposed: a positive control (PC) with nutrient specs as indicated above, a negative control (NC) reducing the PC with 2g Ca and 2 g aP per kg of feed and 4 phytase treatments adding 125, 250, 500 or 1000 OTU OptiPhos® per kg of NC feed. Measured parameters included performance, tibia bone ash content and P levels in tibia bone ash. The results indicated that the reduction of Ca and P led to a significant decrease in end weight (-0.87 kg; $p < 0.05$) and to a numeric increase in FCR. Increasing the doses of OptiPhos® resulted in an increased end weight. OptiPhos® at 1000 OTU/kg gave the same end weight as the positive control. Already at 250 OTU/kg, OptiPhos® reduced the FCR to the original level of the PC, while increasing the dose to 1000 OTU/kg resulted in a FCR which was 0.06 lower than the PC. No differences in tibia ash were found between the PC and the OptiPhos® treatments. Increasing the level of OptiPhos® did result in a phosphorus level of the tibia similar to the PC. Economic calculations indicated that OptiPhos® added at 1000 OTU/kg increased profit over feed by 0.33-0.34 € per bird. It is concluded that OptiPhos® at a dose of 1000 OTU per kg of feed gave a full compensation for the 2 g aP reduction of inorganic P in the feed while reducing the FCR by 0.06 compared to the PC (superdosing effect). The tibia-based result also confirms the matrix value of 1.95 g aP advised for 1000 OTU OptiPhos® per kg feed.

Keywords: phytase, superdosing, OptiPhos, turkey

S1- 0219 OptiPhos® superdosing in broilers shows clear benefits

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A US broiler trial using 1920 Ross- 308 male broilers was conducted to evaluate the effect of 1000 OTU/kg OptiPhos® on top of a standard industry or a feed with minimal P levels on technical and economic performance. The broilers were distributed over 48 pens for 4 treatments, each treatment having 12 replicate pens containing 40 broilers each. Broilers were fed corn/soy based feeds in 3 phases: starter (d0- 14; 3050 kCal ME/kg, 23.4% CP, 1.42 % Lys and 0.95% Ca), grower (d15-28; 3150 kCal ME/kg, 22.8% CP, 1.29% Lys and 0.85 % Ca and finisher (d29-40; 3200 Kcal ME/kg, 19.2% CP, 1.09% Lys and 0.75% Ca). A standard industry feed was produced with these nutrient specs and with available P (aP) levels as applied in practice: 0.5, 0.44 and 0.36% in starter, grower and finisher phase respectively. A second feed closer to minimal P requirements with the same nutrient specs but using aP levels of 0.5, 0.32 and 0.24% in starter, grower and finisher phase respectively was also produced. Both feeds were supplemented with or without 1000 OTU/kg OptiPhos®. The addition of OptiPhos® at 1000 OTU/kg for both types of feed resulted in a significantly increased final weight (+126 g for the industry feed and + 122 g for the feed closer to minimal P requirements), and in a decrease in feed conversion of 0.06 for both feeds. An economic calculation, taking into account a broiler price of 0.8 to 1.2 €/kg live weight and an average feed price of 275 €/T, showed a benefit of up to 142 € per 1000 broilers produced when feeds were supplemented with 1000 OTU/kg OptiPhos®. It could be concluded from this trial that OptiPhos® added at 1000 OTU/kg to a standard industry feed or a feed formulated closer to the minimal P requirements showed an increased end weight by more than 120 grams, reduced feed conversion by 0.06 and yielded a financial gain up to 0.142 € per broiler.

Keywords: OptiPhos®, broiler, phytase, superdosing

S1-0220 OptiPhos® shows superdosing effects in broilers on a feed containing adequate levels of Ca and P

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A broiler trials was set up using 1080 male Ross 308 broilers, distributed over 36 pens, to determine the superdosing effect of 500 and 1000 OTU/kg OptiPhos® phytase on technical and economic performance. Three treatments (each treatment having 12 replicate pens containing 30 broilers each) were subjected to a 3 phase feeding scheme with adequate levels of Ca and P: starter (d 0-13; 0.92% Ca and 0.75% P, grower (d13-26; 0.72% Ca and 0.63% P, and finisher (d26-39; 0.66% Ca and 0.59% P). Control feed was supplemented either with 500 or 1000 OTU/kg OptiPhos®. All parameters were subjected to a factorial analysis of variance and the corresponding LSD- multiple range test. OptiPhos® added at a level of 500 or 1000 OTU/kg increased weight with 65 and 117 g at day 39 respectively, yielding an extra daily gain of +1.9 g/d ($P=0.08$) and +3g/d ($P<0.05$) respectively. Adding OptiPhos® at 500 or 1000 OTU/kg reduced FCR with 0.05 at day 39 for both OptiPhos® levels, and to 0.06 at 500 OTU/kg or 0.07 at 1000 OTU/kg ($P<0.05$) when FCR was recalculated to an adjusted body weight of 2000g. The economic calculation demonstrated an extra gain over feed cost of 57 to 87 EUR for 500 OTU/kg, and of 72 to 119 EUR for 1000 OTU/kg OptiPhos® supplementation on a 1000 broilers scale, depending on the broiler prices varying from 0.8 to 1.2 €/kg). It can be concluded from this trial that OptiPhos® added at 500 and 1000 OTU/kg to a feed containing sufficient amounts of Ca and P increased weight up to 117 g at day 39, reduced FCR up to 0.07 and improved economics up to 0.12 euro per broiler

Keywords: OptiPhos®, broiler, phytase, superdosing

S1-0221 OptiPhos® superdosing improves growth and reduces mortality in broilers in Russian field conditions

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A field study was set up on two well managed broiler farm to evaluate the superdosing effect of OptiPhos® at 1000 OTU/kg on technical and economical performance. One farm had 200.000 birds divided over several houses, while the other farm had 300.000 birds divided over several houses. Two feeds were applied: a control feed being a standard feed with the normally used amount of 250 OTU OptiPhos® per kg feed replacing 0.12% of available phosphorus (aP), and a treatment feed being equal to the control feed however with an additional 750 OTU OptiPhos® added on top (so 1000 OUT/kg feed total) however without lowering the aP further. Two cycles were run after each other, where treatments were exchanged between the sites to balance the trial. The technical results achieved with the control feed (OptiPhos® at 250 OTU replacing 0.12% of aP), were considered good compared to the normally achieved results in this farm (growth 57.4 g/d in 42 days with feed conversion of 1.65). However when 750 OTU OptiPhos® was added extra per kg of feed, the average daily gain increased with from 57.4 to 59.4 g/d resulting in 67 g of extra end weight despite the earlier slaughter age (0.35 days earlier compared to the control birds). Correcting for this difference in slaughter age, a difference of 88g of live weight between control (250 OUT/kg) and treatment (1000 OUT/kg) was observed. Also mortality was reduced from 33 to 2.7 %. An economic calculation assuming a feed price of 250 €/T, a day-old chick price of 0.3 € and a broiler selling price of 1 €/kg live weight showed a net extra profit of €30 per 1000 broilers when OptiPhos® was superdosed at 1000 OTU/kg of feed. It can be concluded that OptiPhos® added at 4 times the normal dose while replacing 0.12% of available P resulted in 2 grams extra average daily gain, 0.6 % lower mortality and an improved economics of 30 euro per 1000 broilers

Keywords: OptiPhos®, broiler, phytase, superdosing

S1- 0222 Superdosing OptiPhos® brings consistent benefits in growth, feed conversion and finances

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Phytate is naturally present in raw materials like corn, soya and wheat and acts as an Anti-Nutritional Factor (ANF) binding minerals, amino acids, fatty acids and even to starch. As all these valuable nutrients are bound to phytate, they become unavailable for the digestive system of the animal and will lead to underperformance of the flock. The hypothesis of superdosing is by applying a phytase in a higher dose than usually for plain phosphorus release (so-called superdosing), these nutrients become available for digestion by the animal. Several field studies have been set up in the period 2012 to 2015 at trial facilities in South Africa, the Netherlands, USA, Belgium and Russia, looking at the effect of superdosing of the phytase OptiPhos® on technical and financial performance. Control feeds adequate in available phosphorous and Ca, either by use of inorganic phosphates or by the inclusion of a single dose of OptiPhos® (250 OTU/kg) were supplemented with OptiPhos® to reach levels of 500 to 1000 OTU/kg without removing additional P from the diet. Average results of these trials indicated that birds grown on feed superdosed with OptiPhos® had a reduced feed conversion of 0.03 to 0.05, and an increased extra weight of 55 to 98 grams, at 500 or 1000 OTU/kg respectively. This equals to an increased body weight gain of 2.5 and 4.3 %, and a reduced feed conversion of 1.8 and 3.2 % at 500 and 1000 OUT/kg of OptiPhos® respectively. A net financial benefit of 5000 to 9600 € per 100.000 broilers slaughtered could be calculated at an OptiPhos® inclusion of 500 and 1000 OTU/kg respectively (considering an average weight of 2.5 kg at slaughter, a feed conversion of 1.70, a feed price of 250 €/T and a life bird price of 1 €/kg). So it can be concluded that superdosing of OptiPhos® up to 1000 OTU/kg has a strong technical and financial effect on broiler production, providing a benefit of 5 to 9.6 eurocent per broiler.

Keywords: OptiPhos®, broiler, phytase, superdosing

S1-0223 Hostazym® X enables the replacement of soybean protein by other protein sources in layer feed, without compromising animal performance

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A layer trial using 432 hens was conducted to evaluate the effect of replacing soybean protein by other protein sources, with or without Hostazym® X at 1500 EPU/kg. Layers were distributed over 3 treatments which each 8 repetitions of 18 animals. An adaptation period until 70% of lay performance was followed by 4 testing periods of 28 days each. Treatments were: a control feed (2750 kCal ME lay, 15% CP and 0.57% dig. Lys.), an alternative feed with the same specs but replacing soybean protein by increasing levels of sunflower meal (from 6 to 8.2%) and rapeseed meal (from 7 to 15%), and the later feed supplemented with Hostazym® X to yield 1500 EPU/kg. Technical performance was monitored weekly, while egg parameters (cracked and dirty eggs) were measured daily. Hens were weighed individually at the start and the end of the trial. All parameters were subjected to a factorial analysis of variance and the corresponding LSD-multiple range test (with $P \leq 0.05$ as the threshold value). Results showed that replacing soy protein sources by sunflower and rapeseed meal decreased laying rate by 1.5%, daily egg mass by 1.3 g ($P < 0.05$) and increased the feed conversion ratio by 0.04. Adding Hostazym® X to this feed brought performance back to, or even above, the level of the control group. Supplementing the trial feed with Hostazym® X led to a relative reduction in cracked eggs by 27% (-0.72% on total eggs) whilst percentage of dirty eggs stayed low. Hen growth during the trial period was 81 g, 98 g and 126 g for the control group, the trial feed and the trial feed + Hostazym® X respectively. An economic calculation showed a high benefit when adding Hostazym® X, reaching up to 17 € per month per 1 000 layers. It can be concluded from this trial that replacing soy protein sources by higher levels of sunflower and rapeseed meal reduced performance but that Hostazym® X added at 1 500 EPU/kg compensated fully for this loss of performance

Keywords: Hostazym X, NSP, xylanase, broiler, protein

S1-0224 Pronutrients use in poultry nutrition

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The objective of the study was to experimentally confirm at a cellular level the epigenetic effects of the inclusion of pronutrients in poultry nutrition. The experiment was designed to prove the hypothesis that pronutrients, which are botanical bioactive molecules, can improve cells physiology by increasing gene expression when included in feed. Consequently, there is more specific proteins generation in ribosomes and therefore an improvement of the target cells functionality. The products tested, Alquernat Neb sui (natural growth promoter) and Alquernat Immuplus (natural immunobooster), are based in pronutrients and therefore expected to stimulate protein synthesis by increasing gene expression. A treatment with cycloheximide was used as a negative control of the technique used, as the inhibitory activity of this compound should correspond to a very weak signal or no signal at all. The experimental design was based on the use of puromycin as a way to compare protein synthesis rates between the different treatments. Materials used include IPEC-J2 and 3D4/2 cell lines, Dulbecco's Eagle medium, puromycin, cycloheximide, Alquernat Neb sui, Alquernat Immuplus, Kerafast Anti-puromycin antibody and Thermo Rabbit Anti-mouse HRP. IPEC J2 and 3D4/2 cell lines were cultured with medium supplemented with the corresponding treatments. After the culture, the samples were then loaded on a SDS-polyacrylamide gel to perform western blot assays, with an anti-puromycin antibody. Results. Revealed of the gels showed that RNA-protein translation rates are much higher in presence of both Alquernat Neb sui and Alquernat Immuplus, in comparison to all other treatments. Alquernat Neb sui and Alquernat Immuplus have an inducing effect on RNA-protein translation rate of enterocytes and macrophages, respectively. This confirms the zootechnical results of preclinical and clinical studies where FCR and growth were improved, without using chemicals.

Keywords: pronutrients, epigenetics, functional proteins, botanical AGP substitutes, natural immunobooster

S1-0226 Effects of L-carnitine supplementation on performance parameters, blood gases and pulmonary hypertension syndrome in the broilers grown at high altitude

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The objective of this study was to investigate the effects of L-carnitine as a potential means of reducing the incidence of ascites in broilers and its relationship with physiological and biochemical parameters. One-day-old 300 male broiler chicks (Ross 308) were used in the trial. The group without L-carnitine supplementation (0) was assigned as control and the groups that received 100, 150, 200 ve 250 mg/l L-carnitine supplementation in water were assigned as treatment groups. The trial was completed in 35 days. At the end of the trial, L-carnitine supplementation did not have any significant effect on live weight gain, feed consumption, feed conversion ratio and water consumption. Levels of blood plasma and hemogram parameters HDL (High Density Lipoprotein), Triglyceride, CK (Creatine Kinase), RBC (Red Blood Cell) and MCH (Mean Corpuscular Hemoglobin) were significantly affected by L-carnitine supplementation ($P<0.05$). Blood gas parameter pH value was significantly affected by L-carnitine supplementation in the broilers with ascites. pH value significantly increased with 100 mg/l L-carnitine supplementation compared to that of control ($P<0.05$). pCO_2 , pO_2 , Hematocrit, Na, K, Ca, Cl concentrations were not significantly affected by L-carnitine supplementation. At the end of the trial, ascites mortality rates starting from the control group were calculated respectively as; 20.00% , 18.33% , 26.67% , 28.33% and 76.71% of ascites deaths were in the 5th week. It was concluded that low doses of L-carnitine supplementation may have positive effects in the broilers grown at high altitude.

Keywords: broiler, L-carnitine, performance parameters, blood gas, pulmonary hypertension

S1-0227 Effect of the oregano essential oil in the improvement of growth performance and digestive tract of broiler chickens

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The present study was designed to evaluate the effect of oregano essential oil on the growth performance and digestive organs weights of broiler chickens. A total of 36 1-day-old broilers (Arbor Acres) were divided into 2 experimental groups; the first group served as control, received a basal diet based on corn and the second group received the same diet, supplemented with oregano essential oil via their drinking water at an inclusion rate of 150 ml per 1000 liters of drinking water, from days 1 to slaughter at day 42. Body, liver and proventriculus weights, size and the weight of the three small intestine portions (Duodenum, Jejunum and Ileum) were measured at 15, 28 and 42 day. Experimental data were statistically analyzed using the t-Student test. Our data revealed that the oregano essential oil did not significantly affect ($P>0.05$) the body weight development, size and weight of the three segments of broiler small intestine, throughout the experimental period. On the other hand, liver and proventriculus weights did not show any significant ($P>0.05$) variation in comparison of the two studied groups. The essential oil of oregano supplementation could be an interesting alternative to antibiotics in the improvement of the broiler's growth.

Keywords: broiler chicken, oregano essential oil, growth performance, digestive tract

S1- 0228 The effect of whole rapeseed on broiler chickens performance gizzard weight and nutrient digestibility

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It has been demonstrated that its feeding value could be affected by incomplete rupture of the seed structure during feed processing but course material improve gizzard functioning. The aim of the study was to investigate the effect of whole rapeseed on gizzard weight and nutrients digestibility. The experiment was conducted with 160 broiler male chickens, divided into two dietary treatments (finely ground rapeseed (FG) or intact seeds (IS)), 14 replications with eight birds in each. The fine material was achieved by grinding the rapeseed using Skiold disc mill (Skiold A/S, Denmark). Content of FG or IS in diet amounted; 5% (8- 21d), 10% (22- 28d) and 15% (28- 35d). Irrespective of incorporated form of rapeseeds (EG or IS) birds were characterized by similar body weight gain ($P<0.36$), feed intake ($P<0.19$) and FCR ($P<0.7$). Gizzard weight at 35 d of experiment, increased when IS where used ($P<0.0013$), (FG; 1.784 vs. IS; 1.981 % of BW). There was no differences in apparent ileal crude fat digestibility. Use of intact seeds improved ileal crude protein digestibility (0.677 vs. 0.751, $P<0.0063$) and tend to increase ileal digestible energy content (12.3 vs. 12.85 MJ/kg of diet, $P<0.0508$). The present trial demonstrates that whole rapeseed in diet had a greater effect on gizzard weight than finely ground seeds.

Keywords: rapeseeds, gizzard, digestibility, protein, fat

S1- 0229 Optimum dietary protein levels based on maximum return on feed cost per body weight gain of birds raised sex separate and straight-run

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Economic benefits of raising birds sex separate (SS) vs. straight-run (SR) has been a paradigm for the broiler industry over the years. Three experiments (exp.) were conducted to evaluate the effects of raising birds SS vs. SR (Exp. 1), and determine optimum digestible lysine (dLys) for the starter (Exp. 2) and grower phases (Exp. 3) for each system. For each of the 3 experiments an economic simulation of net returns of feed cost over live weight was made for different market weights (MW) considering a 1.2 million broiler complex. Data were modeled using various non-linear models to attain the best economic output. Exp. 1 revealed economic advantages of SS rearing, on 1344 broilers chicks of two genetic strains processed at 1.8, 2.8, and 3.8 kg MW, of \$54,348 for Ross 308, and \$80,474 for Ross 708. In exp. 2 3240 Ross 708 chicks were fed starter diets (0 to 25d) with 6 dLys levels (1.05% to 1.80% of dLys) maintaining the amino acid. For a MW of 1.6kg, feeding 1.05% dLys diets resulted in higher profits with the SS birds having an extra income of \$30,094 when comparing with SR. The exp. 3 had 2160 Ross 708 chicks fed grower diets (14 to 32d) with 6 dLys levels (0.90% to 1.30% of dLys). There were 2 projected MW 1.8 kg (females SS and SR mixed sex birds) and 2.8 kg (males SS and SR mixed sex birds). For 1.8 kg MW birds, 0.9% dLys proved to be the most profitable for both rearing systems. Conversely, for 2.8kg MW, feeding 1.3% of dLys resulted in higher returns for both rearing systems. The net return for both rearing systems for both MW showed that SS returned extra \$75,463 comparing to SR. In conclusion, optimum dLys level shown to be dependent on final MW, and that raising birds SS invariably resulted in higher net returns of feed cost over live weight.

Keywords: sex separate, straight-run, broiler, economic, protein

S1- 0230 Positive effect of extrusion cooking on nutritional value of pea seeds for broilers chickens

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The study was conducted to investigate the nutritional value of raw and extruded five different batches of pea seeds. The study group included 110 male one-day-old Ross 308 chickens. The birds were randomly assigned to eleven dietary treatments (10 replications in each). Birds were kept in individual cages. Digestibility was calculated using the difference method. From day 1 to 16, birds received only basal diets. From day 17 to 21, in ten of the treatments, diets contained pea meal in raw or extruded form and the basal diet in the ratio 20:80. On days 19 and 20, excreta were individually collected, and then all chickens were sacrificed and ileal digesta were sampled for determination of ileal digestibility. Extrusion cooking of pea seeds decreased concentration of crude fiber, ADF, NDF, trypsin inhibitor and phytic P as well as resistant starch. ($P \leq 0.05$). Extrusion improved the AMEN values of raw pea seeds ($P \leq 0.05$). The AMEN value of extruded pea was approximately 2,25 MJ/kg DM higher in comparison raw seeds. Extrusion cooking of pea seeds significantly improved dry matter and crude protein digestibility ($P \leq 0.05$). The digestibility of dry matter and crude protein was about 21.3% and 11.6 % higher compared to raw seeds. The similar result was confirmed with respect to digestibility of all analysed amino acids.

Keywords: pea, phytic P, extrusion, anti-nutritional

S1-0231 Egg shell membrane and its potential to modify health and immunity of post hatch poultry

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Eggshells are the byproducts of poultry industry which largely consist of calcareous shells, shell membranes, and adsorbing proteins of embryonic origins. Mass spectrometry analyses of the membrane extracts revealed the presence of over 250 proteins belonging to antimicrobial, enzymatic, cell-regulatory, and organizational genre with hatchery egg shell membranes (HESM) further enriched with many blood derived proteins. The potential of these membranes as feed supplement, however, has not been much explored although many of the membrane bound bioactive factors can provide physiological benefits in the paradigm of enzyme, antibody, or cytokine micro supplements provided in animal feed. Thus, the objective of this study was to explore and evaluate the potential of egg shell membrane(ESM) supplements on growth and physiological variables of post hatch chickens under normal or endotoxin challenged conditions, measured at 2 different ages, 3 and 5 weeks, respectively. Unfertilized and hatchery ESM were pulverized to powdery flakes, and mixed with feed either intact or after alcohol decontamination at the concentrations ranging between 0.2 to 0.5% (w/w) and given to chickens for first 2 weeks post hatch and evaluated at 3 and 5 weeks respectively. Chickens receiving both types of ESM supplement showed slight to moderate improvement of growth and age dependent changes in serum antibody levels following treatment, decreased stress variables such as corticosterone and heterophil/lymphocyte ratios. When challenged with bacterial lipopolysaccharide (LPS), the HESM fed chickens showed substantial protection against body weight loss compared with controls, decreased upregulation of pro-inflammatory cytokine genes while favoring the upregulation of anti-inflammatory genes. Thus, our results show that ESM supplement during post hatch development can improve immunity, resistance to endotoxin, and decrease stress variables suggestive of its potential benefit to the bird.

Keywords: egg shell membrane, feed supplement, immunity, stress

S1- 0232 Cholesterol metabolism changed at the early stages of ascites syndrome in broiler chickens

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Cholesterol is essential for life, and disorders related to cholesterol metabolism may become metabolic diseases. To investigate the changes that occur in cholesterol metabolism in broiler chickens with cold-induced ascites syndrome (AS), 216 one-day-old male broilers were randomly assigned to two groups (control and AS), with six replicates of 18 birds each. Starting on day 14, the birds in the AS group were exposed to low ambient temperatures (17 °C during the daytime and 14 °C at night) to induce AS. The serum total cholesterol (TC) content of the AS group was significantly increased ($P < 0.01$) at day 21; however, there was no significant difference between groups at day 35. Additionally, there was no significant difference in the hepatic mRNA expression of 3-hydroxy-3-methyl-glutaryl-CoA reductase, low-density lipoprotein receptor, apolipoprotein AI, ATP-binding cassette transporter G5 and ATP-binding cassette transporter G8 at days 21 and 35. The hepatic mRNA expression of cholesterol 7 α -hydroxylase (CYP7A1) and liver X receptor α of the AS group was significantly decreased ($P < 0.05$) at day 21, whereas there was no significant difference at day 35, as was the hepatic CYP7A1 protein content. The results of this study indicate that the increased serum TC observed in the AS group at day 21 may be the result of unchanged cholesterol synthesis, uptake, transport and reduced cholesterol decomposition in the liver; the unchanged serum TC content observed in the AS group at day 35 may be the result of unchanged cholesterol synthesis, uptake, transport and decomposition in the liver.

Keywords: cholesterol metabolism, ascites syndrome, broiler chickens, liver

S1-0233 Effect of dietary high level of manganese on the immune response of broilers against Salmonella Typhimurium challenge

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To investigate the effects of dietary high level of manganese (Mn) on the immune responses of broilers challenged with Salmonella Typhimurium, 144 1-day-old male Cobb broilers were randomly divided into two groups and fed with corn-soybean basal diet supplemented with 60 mg Mn/kg (NRC recommended level, Control) or 500 mg Mn/kg (high level, H-Mn). At 7 days of age, half of broilers in each group were respectively orally inoculated with Salmonella Typhimurium ATCC 14028 (5×10^7 cfu) or LB broth. The birds ($n=6$) were sacrificed at 2 and 7 days post-inoculation (DPI). Plasma lymphocyte subsets were determined via flow cytometric analysis. The cecal tonsil, spleen and bursa of Fabricius were collected for mRNA expression through qPCR, and the jejunum was collected for morphological determination. At 2 DPI, H-Mn increased ($P < 0.05$) the CD3+CD4+ and CD3+CD8+ percentages in inoculated broilers, while did not alter CD3+CD4+ percentage and decreased CD3+CD8+ percentage in non-inoculated broilers. At 7 DPI, Salmonella inoculation decreased CD3+CD4+ percentage in broilers despite of Mn levels, whereas only increased CD3+CD4+/CD3+CD8+ ratio in broilers fed with control diet. The Salmonella inoculation increased ($P < 0.05$) jejunal villus/crypt ratio in birds fed with control diet at 2 DPI, but in birds fed with H-Mn diet at 7 DPI. At 2 DPI, H-Mn increased IL-6, IL-8, IFN- γ mRNA levels compared with control in inoculated birds, while decreased IL-8 and IL-17 in non-inoculated broilers. H-Mn increased IL-6 and IL-8 in bursa of Fabricius compared with control in non-inoculated birds, not in inoculated birds at 7 DPI. In the cecal tonsil, H-Mn significantly decreased IFN- γ and NF- κ B whether inoculated or not at 2 DPI, but only increased IL-17 in inoculated broilers, not non-inoculated broilers. The results suggested that dietary Mn level influenced the immune responses of broilers against Salmonella challenge through regulating the Th1/Th2 cytokines expression in different tissues.

Keywords: Salmonella Typhimurium, Manganese, immune responses

S1-0234 Determination of energy equivalency of lysophospholipids in broiler diets

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An experiment was conducted to determine the energy equivalency of lysophospholipid in broiler diets. Experiments were carried out as a completely randomized design involving 9 treatments of 6 replicates and 10 chicks in each pen. BW and cumulative feed intake were measured weekly. Lipid profile, plasma calcium and phosphorus and relative organ weight and length were measured at 42 d of age. Replacement of soybean oil with lysophospholipid severely inhibited BW gain, also increased FCR without any consequence in feed intake in broilers fed soy-corn mash diets ($P \leq 0.05$). Lysophospholipid supplementation did not significantly alter blood lipid profile and plasma phosphorus and calcium content, and HDL and LDL cholesterol concentration. The inclusion of lysophospholipid linearly ($P \leq 0.05$) increased abdominal fat content of broilers fed mash diets. Nonlinear and linear equations were generated for the lysophospholipid and graded levels of Energy in mash diets. Based on an assessment for the r^2 and P values of the equation, for body weight gain, feed intake and feed conversion ratio were sensitive measurements of responses to energy levels in treatments. The nonlinear or linear response equation with the higher r^2 values for added lysophospholipid and the equation for different levels of energy were set to be equal and were solved. The body weight gain and feed conversion ratio responses from each gram of lysophospholipid per kg of diet at 28 and 42 d of age were therefore equal to 6, 9, 4.7 and 7 kcal/kg of diet respectively.

Keywords: lysophospholipid, energy, performance

S1- 0235 Impact of Neem (*Azadirachta indica*) seed extract on the growth, haematological parameters and serum cholesterol of broiler chicks

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The impact of Neem (*Azadirachta indica*) seed extract (NSE) on the growth, haematology and serum cholesterol of broiler chicks was evaluated in a study that lasted 35 days. 225, 7 day old broiler chicks were randomly assigned to five treatments which contained 0, 15, 30, 45 and 60ml NSE per litre of water representing T1, T2, T3, T4, and T5 respectively in a completely randomized design experiment. Each treatment was replicated three times with 15 birds in each replicate. Feed and water were given ad libitum. Data collected on growth, haematology and serum cholesterol parameters were subjected to analysis of variance. There was no significant ($P > 0.05$) difference in weight gain, feed and water intakes, feed conversion and protein efficiency ratios of the birds among the treatments. NSE had significant influence ($P < 0.05$) on the packed cell volume, red blood and white blood cells. There was no significant ($P > 0.05$) difference in the haemoglobin and serum cholesterol of the birds among the treatments. In conclusion, results of this study indicate that NSE could therefore be used in broiler production without any deleterious effect on the growth, haematology of broiler chicks.

Keywords: *Azadirachta indica*, broilers, growth, haematology, serum cholesterol

S1- 0236 Supplementation of processed plant protein in diet and as paste improves performance of broiler chickens

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This study aimed to determine the effect of route of supplementation of a high quality processed plant protein – HP Avistart (HPA, Hamlet Protein) on the performance of broiler chickens. A total of 252 day-old Ross 308 chicks (male) were randomly placed on four treatments (control diet, control diet + HPA paste for 5 days, diet with 10 % HPA only and diet with 10 % HPA + HPA paste for 5 days) for 10 days in 7 replicates of 9 birds each. Thereafter, the birds were fed common grower and finisher diets at each growth phase. Feed and water were provided ad libitum. On days 5, 10, 24, and 35, feed intake and body weight (BW) were recorded. Results on d5 showed that groups on control and 10 % HPA only diets were significantly ($P < 0.05$) higher in BW gain and feed intake. Feed conversion ratio (FCR) was significantly ($P < 0.05$) higher in the two groups without paste supplementation. On d10, the control group was significantly ($P < 0.05$) different from the 10 % HPA + HPA paste group in feed intake, while no difference was observed in BW gain and FCR between other groups. On d24, group fed 10% HPA only was significantly ($P < 0.05$) but with marginally higher FCR in control group. On d35, groups with HPA in diet and/or as paste were significantly ($P < 0.05$) different from the control group in BW gain. We conclude that supplementing processed plant protein paste, in addition to in-diet inclusion may contribute to a positive significant effect on broiler chicken growth responses. Such a paste may be beneficial for use in in-hatchery or on-site early feeding systems.

Keywords: plant protein, performance, weight gain, feed intake

S1-0237 Effects of green and oolong tea powder supplementation on broiler growth performance, and intestinal characteristics

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The aim of this study was to investigate the effects of dietary green tea and oolong tea powder supplementation on broiler growth performance, antioxidant activities and intestinal characteristics. Proximate analysis on green tea and oolong tea powders were done in the lab to measure the total phenolic content, TEAC antioxidant effects, DPPH radical scavenging effects, H₂O₂ chelating effects, crude protein content, ash, acid detergent fiber and neutral detergent fiber respectively. The experimental design consisted of a total number of mixed-sex 240 day-old ROS 308 chicks (120 males and 120 females) allocated to three treatments with four replicates containing 20 birds each. The dietary treatments consisted of 0.5% green tea powder, 0.5% oolong tea powder and control. Blood collection from the wing vein was done at days 19 and 31 for catalase and malondialdehyde determination. Body weight and feed intake of broilers were measured at 1, 21, and 35 days, and the feed conversion ratio were calculated accordingly. At days 21 and 35 four birds from each replicate were slaughtered and organs (intestines, liver, spleen, and heart) were weighed. Intestinal contents were emptied into containers for microbial counts. The supplementation of 0.5% green and 0.5% oolong tea powder had no significant effect on broiler body weight gain. However the supplementation of 0.5% green tea and 0.5% oolong tea powder decreased caecal coliform count ($P<0.01$) and improved blood serum antioxidant activities by increasing serum catalase concentration ($P<0.01$) and decreased serum malondialdehyde ($P<0.01$). In conclusion, the results obtained from this research study had shown that the supplementation of 0.5% green tea and 0.5% oolong tea powder in broiler diets from 1 to 35 days could decrease cecal microbial counts and increase blood serum antioxidant activities.

Keywords: green tea, oolong tea, catalase, malondialdehyde, micro flora

S1-0238 Effect of the different feed forms on egg production and incubation results in Ostrich (*Struthio camelus domesticus*)

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This research was carried out to determine the effects of different feed forms on egg production and incubation results during the breeding season of ostrich (*Struthio camelus domesticus*) from 2012 to 2013. Breeding ostriches were fed with pelleted feed in 2012 (1st breeding season) and mash feed in 2013 (2nd breeding season). In the experiment, each of feed (pelleted and mash) had similar metabolic energy and crude protein content (18% CP, 2450 kcal ME). The effects of different feed forms on number of laid eggs per female, average egg weight and length of laying period were found to be significant ($P<0.01$). The number of laid eggs per female per season was 44.7 in the 1st breeding season whereas this number decreased to 39.6 in the 2nd breeding season. The length of laying period lasted 185 days in the 1st feeding practice whereas it declined to 180 days in the 2nd breeding season. Average egg weight was found significantly different as 1490.7 and 1280.5 g in 1st and 2nd breeding seasons, respectively ($P<0.01$). On the other hand, different feed forms affected the hatchability of total eggs, hatchability of fertile eggs, chick weight at hatching, fertility, malpositioned embryos, deformed chicks and assisted chicks during hatching ($P<0.01$). Hatchability of fertile eggs decreased from 71.7% to 62.7%, whereas middle term embryonic mortality increased from 4.5% to 10.3%, one from the other breeding seasons. These results showed that mash feed form had negative effects on egg production parameters and incubation results.

Keywords: Ostrich, breeders, nutrition, feed form, egg production, incubation

S1-0239 Effects of a selected formula of protected organic acids and essential oils on digestive enzyme activities and intestinal microflora in broilers

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This experiment was conducted to investigate the effects of protected essential oils and organic acids on digestive enzyme activities and intestinal microflora in broilers. 450 one-day-old Cobb 500 chicks were randomly allotted into three treatments with 6 replicates per treatment and 25 birds per replicate. The dietary treatments were as follows: control group (basal diet), antibiotics group (control + 0.15 g/kg enramycin) and feed additive group (control + 0.30 g/kg protected organic acids and essential oils). At the age of 21 and 42 day, digestive enzyme activities were examined in duodenum, jejunum and ileum. Microflora in duodenum, jejunum, and ileum were analyzed by polymerase chain reaction (PCR)-denature gradient gel electrophoresis (DGGE). Results have shown that there were no differences in the amylase activity of intestinal tract among three groups ($P > 0.05$), but trypsin, chymotrypsin and lipase activities of intestinal tract were increased in the feed additive group when compared with the control ($P < 0.05$). PCR-DGGE sequence analysis indicated that feed additive supplementation modulated the composition of microbiota mainly by changing intestinal tract Lactobacillus. These results suggested that dietary addition of a selected formula of protected organic acids and essential oils could increase digestive enzyme activities and modulate the gut microbiota.

Keywords: essential oils, organic acids, gut health, microbiota, broiler

S1-0242 The probiotics research distribution and trend in poultry

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Probiotics has been in top list of antibiotics alternatives in last decades. In definitions, probiotics are live microbial feed additives which have positive effects on the host animal mainly through improving the intestinal microbial balance". Since 1990s the scientists triggered huge effort to find the new probiotics and test their effects on the poultry performance and health. However the researches in probiotic field have followed different trends based on the economical condition of countries. Based on the statistic extracted from the Pubmed data bank in the period of 1990 to 2015, 486 paper has have been published in the field of probiotic application in poultry. In 1990s, 29 publication was carried out in this area which increased to 174 publication in 2010s and 283 in the mid of the 2010s. In both the 1990s and 2000s, 65% of the publication was carried out in developed countries; however since 2010 until now, 54% of the the probiotic related researches done in the developing countries. The publications distribution for different geographic areas, North America, Western Europe, developed Asian Pacific countries, China, South America, Eastern Europe, Other Asian countries and African countries were as follow, 1990s: 30, 16, 4, 0, 15, 18, 11 and 4; 2000s: 31, 27, 8, 3, 10, 9, 9 and 3; 2010s: 22, 19, 10, 15, 6, 10, 17 and 1 percent. The Lactobacillus has been the subject of almost 50% of the researches, followed by Bacillus 23%, Enterococcus 11%, Saccharomyces 6%, Bifidobacterium 4%, Pediococcus 3% and other species 3%. This survey confirms that after almost three decades, the probiotics is still an attractive topic in poultry researches. However a shift in the origin of researches from developed countries to the developing countries in 2010s is noticeable. Especially the China has been an important country in this respect so that its share in this area from almost zero in 1990s, has increased to 15% in 2010s.

Keywords: probiotics, geographical distribution, trend and statistic

S1-0243 Investigation the dietary effects of probiotic, prebiotic, and their combination on performance, immune response, tibia bone and excreta characteristics in Japanese quail

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This experiment conducted to investigate the effects of probiotic, prebiotic and their combination in Japanese quail ration on performance, immune response, tibia bone and excreta characteristics. 540 one-d old mixed sex Japanese quail were used in 9 treatments with 4 replicates and 15 chicks per each from 1- 50 days of age. Chicks ration supplemented with 100 and 90% of recommended levels of additives. The results showed that in finisher phase, probiotic supplementation in whole level decreased body weight gain in comparison to other groups ($P<0.05$). Feed intake in total rearing phase affected by probiotic level indicated that whole level of probiotic decreased FI in relation to deficient level significantly ($P<0.05$). Also, deficient level of probiotic led to improve of FCR ($P<0.05$). There were no significant difference in immune response of chicks in prebiotic consumed group at 35 days of age ($P>0.05$). But, in this treatment, tibia length increased significantly in comparison to whole prebiotic level ($P<0.05$). Also, with increasing level of probiotic pH of duodenum and jejunum increased significantly compared to no probiotic group ($P<0.05$).

Keywords: probiotic, prebiotic, synbiotic, performance, immune response, tibia bone

S1-0244 Study on the effects of Salvia Mirzayanii essence levels on immune response parameters, tibia bone characteristics and blood biochemical of broilers under heat stress

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This experiment was conducted to evaluate different levels of Salvia Mirzayanii essence on some blood biochemical and immune response of broiler chickens under heat stress as a completely randomized design with 4 treatments, 5 replicates with 12 chickens per replicate for 42 days. Increasing levels of Salvia Mirzayanii essence (0, 150, 300, 450 ppm) were used. Heat stress was applied for 22 days. At 14 and 35 d. of age, 2 birds per replicate selected and infused by SRBC and 7 days after, blood samples taken for immune response and biochemical assays. Also, at the end of experiment, 2 chicks per replicate randomly selected and slaughtered for tibia bone characteristics. The results showed that percentage of heterophil decreased and % of monocyte increased by increasing levels of essence at 21 days of age ($P<0.05$). But, there was a significant difference between treatments after heat stress at 42 d. of age. Also, glucose, triglyceride, HDL and LDL amount was not significantly affected by experimental treatments ($P>0.05$). Highest weight and ash of tibia bone was observed at 450 ppm essence that has significant difference with control group ($P<0.05$).

Keywords: salvia mirzayanii, heat stress, blood parameters, tibia, immune response

S1-0245 Effects of different levels of natural glauconite and zeolite on performance, tibia bone characteristics and blood parameters of broiler chicken

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An experiment with 300 one-day old Ross male broiler chicks was conducted to determine the effects of glauconite and zeolite on the broiler's performance, tibia bone and blood characteristics. Five experimental treatments [control, glauconite (2 & 4 percent), and zeolite (2 & 4 percent)] were used in a completely randomized design with 4 replicates. During the experiment weight gain, feed consumption and feed conversion ratio (FCR) were measured periodically. At 42 days of age, one chick per replicate was slaughtered to determine calcium and phosphorus of blood serum and tibia bone parameters. Analysis of variance and separation of means by Duncan's multiple range tests were conducted by SAS software. The results indicated that by adding 4% zeolite to diet, weight gain was increased in starter and total rearing period ($P < 0.05$). In comparison with other treatments, feed consumption at 0-42 days of age was increased significantly ($P < 0.05$) in 4% zeolite treatment except 4% glauconite group. There were no significant differences in FCR among treatments ($P > 0.05$). Also, adding 4% zeolite led to significantly ($P < 0.05$) increase in tibia bone volume compared to 2% zeolite group but experimental diets had not significant effect on tibia bone relative weight, length and density ($P > 0.05$). Supplementation of diets with glauconite and zeolite did not have effect on serum Ca and P content at the end of experiment ($P > 0.05$).

Keywords: broiler, glauconite, zeolite, performance, tibia

S1-0246 Efficacy of natural zeolite and glauconite dietary supplementation on carcass characteristics, gut pH and performance of broiler chickens

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An experiment with 300 one-day old Ross male broiler chicks was conducted to determine the effects of glauconite and zeolite on the broiler's carcass characteristics, gut pH and performance. In this experiment, glauconite added for first time in broiler diet as new feed additive. Five experimental treatments [control, glauconite (2 & 4 percent), and zeolite (2 & 4 percent)] were used in a completely randomized design with 4 replicates. During the experiment energy efficiency ratio (EER), protein efficiency ratio (PER), European efficiency ratio (EEF) and European broiler index (EBI) were measured periodically. At 42 days of age, one chick per replicate was slaughtered to determine carcass characteristics and gut pH. Analysis of variance and separation of means by Duncan's multiple range tests were conducted by SAS software. The results indicated that adding zeolite and glauconite to diet do not affected EER, PER, EEF and EBI at total rearing phase ($P > 0.05$). Also, by adding 4% glauconite to diet, carcass percentage and breast relative weight was increased ($P < 0.05$) in relation to control and 2% glauconite group. For thigh relative weight, there were no significant differences between treatments ($P > 0.05$). Supplementation of diets with glauconite and zeolite did not have effect duodenum, jejunum and ileum pH at the end of experiment ($P > 0.05$). In conclusion, we can use glauconite on broiler diet without any side effect whereas some beneficial effects were seen in carcass characteristics.

Keywords: broiler, glauconite, zeolite, gut pH, carcass

S1- 0249 Effect of dietary oregano extracts on caecum microorganism and immune function in broilers

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The objective of this study was to investigate the effects of Oregano Extracts(OS,phytogen) on caecum microorganism and immune function when used as dietary supplements to the broilers. A total of 2400 1-d-old broiler chickens were randomly assigned to six groups with 8 replicates with 50 chickens each and fed for 42d. Six dietary treatment groups were: basal diet (control; CON); CON + Enramycin 10 g · t⁻¹ and Colistin Sulfate 20 g · t⁻¹ (A); CON + OS100 g · t⁻¹; CON + OS150 g · t⁻¹; CON + OS200 g · t⁻¹; CON + OS250 g · t⁻¹. Blood samples were collected at 21d and 42d respectively. At the end of the experiment, the thymus samples, spleen samples, bursa samples were collected from chicks, chicks sacrificed, and caecum contents, feces samples were used to measurement of microorganism. The chickens that received supplement of OS exhibited significantly higher quality of Lactobacillus and Bifidobacterium in caecum ($P < 0.05$), and lower Escherichia coli in caecum and feces ($P < 0.05$). There is no significant difference between the groups CON and A ($P > 0.05$). OS or antibiotics of broiler chick diets non significantly increased thymus index, spleen index, bursa index when compared with CON ($P > 0.05$). Dietary OS also caused a significant increase in both lymphocyte proliferation rates, LTT(LPS or ConA) or Neutrophil Phagocytosis on days 21 ($P < 0.05$), and a tendency to increase on days 42 ($P > 0.05$) compared with the group control, but had no effect on LTT(LPS or ConA) or Neutrophil Phagocytosis in whole experiment period when compared with A. These results showed OS can be included in broiler chick diet at 150 g · t⁻¹ as diet supplementation and the results strongly indicated usage for improvement micro-ecology environment of bird's caecum to some degree and cell immune level, and has no difference in different sex broiler chickens.

Keywords: oregano extracts; broilers; caecum microorganism; immune function

S1-0250 Effect of phytase on performance, tibia ash and litter quality of broilers

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The objective of the trial was to provide efficacy data of a phytase for use in broilers. For this purpose Natuphos E 5000 was introduced in diets with a reduced nutrient content. The experiment consisted of 12 dietary treatments with each 6 replicates of 30 male birds (Ross 308). The first dietary treatment consisted of a corn-soybean diet which met all dietary recommendations (T1). Four different negative control diets (T2, T5, T8 and T11) were calculated by reducing the nutrient content (metabolic energy (ME), crude protein (CP), amino acids (AA), calcium (Ca) and phosphorus (P)) of the positive control by four different matrix values. Reduction of Ca, P, CP and ME level ranged from 1.50 to 2.55%, from 1.15 to 1.96%, from 2.3 to 3.8% and from 0.22 to 0.38 MJ/kg, respectively. Natuphos E was supplemented at 250 (T3), 350 (T4), 500 (T6), 600 (T7), 750 (T9), 850 (T10) and 1500 (T12) FTU/kg. Performance data, litter quality, feather dirtiness and tibia ash percentage were determined. Reducing the nutrient level in a balanced way without phytase supplementation resulted in significantly lower performance results compared to the positive control group. These diets also had a deleterious effect on bone mineralization as indicated by the significantly lower tibia ash %, indicating that Ca and P levels of these diets were insufficient. Adding phytase could compensate for these shortcomings as supplementation of phytase significantly increased feed intake and growth. Broilers fed T12 (lowest Ca and P level combined with phytase) obtained the best feed conversion ratio of all dietary treatments. No effect on feather dirtiness was observed but litter quality seemed to be better for the negative control diets. This study indicates that supplementing phytase can compensate for lower performance results even if standard Ca and P levels are reduced by 30%.

Keywords: broilers, phytase, performance, litter, bone quality

S1- 0251 Effect of feed form and change of feed form on the performances, mortality and footpad dermatitis of fastgrowing broilers

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The objective of our trial was to judge the effect of feed form and the change of feed form at different ages in very fast growing broilers. Five dietary treatments were compared: “fine” mash (FM), “coarse” mash (CM) and pellets (P) fed from birth until slaughter age. Treatment four (MPP) consisted of “fine” mash both in the starter (0-13 d) and grower period (13-26 d) but pellets during the finisher period (26-39 d). MPP birds on the other hand received mash only during the starter period. In the pelleted grower and finisher diet 1% and 2% fat, respectively, was exchanged with wheat for technological reasons. Each feeding regime was fed to 16 pens with 30 male Ross chicks. Performances and footpad lesions were followed in 8 pens per treatment while the remaining 8 pens of each treatment were additionally used to judge mortality. P-feeding significantly increased feed intake compared to both M-feeding counterparts (+16%, $P<0.01$). This resulted in a final weight at 39 d of 2,967 g; 2,519 g and 2,454 g for P, CM and FM fed birds, respectively. When broilers were given pellets from grower onwards (MPP) feed intake was significantly increased ($P<0.01$) compared with both mash treatments, resulting in a final weight of 2,823 g. MPP birds increased their feed intake to a much lower extent and by consequence their final weight (2,593 g), was not significantly higher than CM-birds. The FCR of MPP-birds was lower (1.497, $P<0.01$) compared with all other treatments. Also the weight adjusted FCR2500 was most favourable in MPP-birds. However, if broilers were fed exclusively pellets, a higher incidence of footpad dermatitis and hock lesions ($P<0.05$) compared with FM, CM and MPP was assessed. Mortality rate was higher in P-birds and amounted 7.5% vs < 3.3% in all other treatments and occurred mainly in the second and third week. Based on the performances, mortality and hock lesions, a transition from mash to pellets at 13 d (MPP) was the best option for quickly growing broilers.

Keywords: broilers, feed form, performances, mortality, footpad dermatitis

S1- 0252 Level of supplementation, but not variety, of double-low rapeseed meal influenced growth performance of broiler chickens

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The objective of the current experiment was to investigate the effect of graduated replacement of maize and soybean meal (SMB) in diets with meals from two contrasting (in terms of glucosinolate) varieties of double-low rapeseed (OSRM) on growth performance and gut characteristics of broilers. A total of 1,350 male broiler chicks were allocated to 10 treatments including a wheat-soybean meal (SBM) control diet and 8 additional diets in which each of the two OSRM was added at the rates of 50, 100, 150 or 200 g/kg to replace partly wheat and SBM. Diet 10 had unprocessed double-low rapeseed (OSR) added at 80 g/kg to partly replace wheat and SBM. All the diets were formulated to be equal in energy, protein and standardised digestible amino acid contents. Each diet had 10 replicates with 15 birds per replicate. Birds and feed were weighed on days 0, 21 and 42 for calculation of growth performance data. One bird per pen was euthanised on day 42 for measurement of length and weight of the small intestine, gizzard, pancreas, and liver. There were no statistically significant effects of OSRM variety or variety \times inclusion level interaction on any of responses. In both the starter (day 21) and finisher (day 42) phases, weight gain decreased ($P < 0.05$) with addition of OSRM, but feed intake, relative to the control, was not affected by OSRM when included at up to 100 g/kg. Consequently negative effects ($P < 0.01$) of OSRM inclusion on FCR was only observed at 150 and 200 g/kg levels. There were no effects on feed intake during the finisher phase. In addition growth performance was not significantly different for birds receiving OSRM at 100 g/kg or OSR at 80 g/kg levels. There were no treatment effects on weight of any of the digestive organs (or the length of the small intestine) in the broilers. It was concluded that the broiler chicks tolerated up to 100 g OSRM/kg and 80 g OSR/kg but higher inclusion levels of OSRM are deleterious to growth performance of broilers.

Keywords: double-low rapeseed meal, inclusion level, broilers, growth performance, gut characteristics

S1-0253 Correlating feed conversion ratio and chemical components of wheat and the influence of xylanase and wheat on nutrient utilisation and whole body energetics in broilers

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The objective of the experiments reported here was to study the effect of the chemical composition of feed wheat samples on broiler FCR. Day old broilers (768) were allocated to 16 treatments (2×8 factorial) in a randomized complete design, with 8 wheat samples and 2 levels of xylanase (0 and 16,000 BXU/kg). Each treatment had 6 replicates with 8 birds each. The wheat soybean meal based diets were formulated to be marginally low in metabolisable energy (ME). Wheats were analysed both chemically and using Near Infrared Reflectance Spectroscopy (NIR). Growth performance data were collected on days 0 and 21, and excreta and ileal digesta were collected on day 24 for calculation of nutrient utilization. Whole body energetics were calculated using the comparative slaughter technique. Analysed and NIR-predicted chemical compositions of wheats were correlated to FCR. There were only significant ($P < 0.05$) wheat effects on ileal dry matter and nitrogen digestibility. Ileal digestible energy was improved ($P < 0.05$) by xylanase supplementation, irrespective of wheat. The effect of xylanase supplementation at the total tract level was dependent on the wheat sample. In general nutrient utilisation and ME were improved more so in those wheat samples having relatively lower digestibility. There were no wheat × xylanase interaction for any of the whole-body energetic responses except for heat production (HP) and efficiency of energy accreted as protein. Xylanase did not influence whole body energetics. The FCR of broilers receiving xylanase-supplemented diets were positively correlated ($P < 0.05$) with wheat phosphorus, fat, NDF, total arabinoxylan (AX), soluble AX, and insoluble non starch polysaccharides ($r \geq 0.68$). The study showed that xylanase reduced the differences between wheats with regards to nutritive value and that the primary drivers of FCR are the components in wheat which are antinutritive in nature.

Keywords: correlation, xylanase, digestibilities, carcass accretion

S1-0254 Effect of soaked false yam (*Icacina oliviformis*) seed meal on egg production of chicken

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False yam (*Icacina oliviformis*) is a drought resistant shrub that grows in the wild in the Savanna regions of West and Central Africa. The seed contains nutrient composition similar to that of maize grain, but contains a bitter principle (resin). Soaking may improve its nutritional value. Soaked false yam seed meal (SFYSM) was prepared as follows: fruits cracked to remove seeds, soaked in water for 12 d and water changed every 3 d, sun-dried (5 d) and milled. It was substituted for maize (w/w) in layer chicken diets at varying levels (0, 25, 50, 75 and 100 g/kg) to determine the effect on egg production. At 20 wk of age, 300 ISA BROWN pullets were placed in deep litter pens and fed one of the 5 diets (6 replicates, 10 hens per replicate) in a Completely Randomized Design up to 38 wk of age. Data were collected on weekly feed intake, egg production variables and mortality; and analyzed by ANOVA using 'GenStat'. Only hens fed SFYSM at 100 g/kg consumed more ($P < 0.05$) feed (98 g/b/d) than the control hens (92 g/b/d). Mean hen-day egg production (75% versus 76-80%), feed/egg mass ratio (2.0 versus 1.8-1.9), egg weight (52 g versus 52-53 g), Haugh Unit (67% versus 61-69%) and egg shell thickness (0.30 mm versus 0.27-0.31 mm) were similar ($P > 0.05$) for all hens. Mortality was recorded only in the control group. It is concluded that SFYSM can be substituted for maize in layer chicken diet up to 100 g/kg without adverse effects on egg production.

Keywords: false yam seed, soaking, egg production

S1- 0255 The effect of feed restriction on changes in carcass composition of males and females of broiler chickens

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The aim of the study was to investigate the effect of two restriction levels and ad libitum feeding on the development of carcass yield in pullets and cockerels of broiler chickens at the end of feed restriction and in the realimentation period. Two-factorial experiment with Ross 308 broiler chickens was split into 6 groups according to feeding regime and gender of chickens. There were three groups of feeding regime, group one was fed ad libitum (ADL) during the whole experiment, group two was restricted 8th and 14th day of age and received 80% of ad libitum (R80) and group three was restricted at the same age and was fed 65% ad libitum (R65). At the end of restriction and then in a week interval till the end of the experiment in 35 days of age, 8 chickens from a group on average weight were selected for slaughter analysis. At the end of feed restriction, the significant interaction of feeding regime and sex was observed in breast percentage ($P=0.05$) with the highest percentage in ADL pullets (22.4%) and the lowest in R65 pullets (18.8%). Breast percentage in restricted males and females significantly decreased ($P=0.001$) and remained numerically lower till the end of the experiment. The significant interaction was also found in meat bone ratio ($P=0.05$) at 14 days of age, at 21 days meat bone ration was lower ($P=0.05$) in restricted chickens compared to the ad libitum ones. At this age, restricted chickens had significantly higher ($P=0.05$) dressing out percentage, abdominal fat percentage ($P=0.05$). Dressing out percentage was significantly ($P=0.05$) affected by interaction of evaluated factors at 21 days of age. Abdominal fat was significantly higher in restricted males and females during the whole realimentation period. Thigh meat percentage was higher in males than in females at 14 ($P=0.05$), 21 ($P=0.001$) and 35 days of age ($P=0.05$). Results of the experiment show that feeding regime and realimentation affected mainly meat bone ration and abdominal fat percentage.

Keywords: chicken, restriction, carcass yield

S1-0256 Use of a phytobiotic supplement to reduce the impact of necrotic enteritis in broilers

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Poultry necrotic enteritis (NE) is caused by *Clostridium perfringens*. The disease may result in devastating economic loss and is mainly controlled by the use of antibiotics at present. Numerous alternatives to in-feed antibiotics have been intensively studied including phytobiotics. The current study examined whether a standardized blend of plant-derived isoquinoline alkaloids (IQ, Sangrovit® Extra) could alleviate the impact of NE in broilers. Ross 308 male broilers ($N=714$) were assigned to 42 pens in a 2 x 3 factorial design. Factors were: NE challenge - no or yes; additives - none, Zn bacitracin (Albac® 150) 0.33 g/kg from d 0 to 35, IQ 0.15 g/kg from d 0 to 35. Broilers were challenged with 1 mL broth with 108 CFU/ml *Clostridium perfringens* after an *Eimeria* inoculation on d 9. The diets were formulated to meet the nutrient requirements recommended for the Ross 308 birds. On d 16, intestinal tissues (duodenum, jejunum and ileum) were scored for NE lesions. Weight gain, feed intake and FCR were determined on d 10, 24 and 35. Performance data were analysed by using a General Linear Model and lesion score by the nonparametric Kruskal-Wallis test. The results showed IQ supplement significantly reduced NE lesion score in challenged birds ($P < 0.05$) and improved weight gain, feed intake and flock uniformity from d 0 to d 35 compared to controls ($P < 0.05$). There was no challenge x additive interaction ($P > 0.05$). The results suggest that the phytobiotic supplement can be potentially used as antibiotic replacement in protection of broilers from NE infections.

Keywords: broilers, necrotic enteritis, phytobiotics, antibiotic replacement

S1- 0257 Effect of *Bacillus subtilis* PB6 on growth and gut microflora in *Clostridium perfringens* challenged broilers

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The objective of current study was to investigate the effect of *Bacillus subtilis* PB6 as a probiotic in broilers. The corn-soybean based diet was divided into four treatment groups; T1 (basal diet with no probiotic and no *Clostridium perfringens*); T2 (basal diet challenged with *C. perfringens* without probiotic); T3 (basal diet challenged with *C. perfringens* having 0.05% probiotic); T4 (basal diet challenged with *C. perfringens* having 0.1% probiotic). Every treatment group had four replicates with 24 birds each. Body weight and feed intake were measured on weekly basis, while ileal bacterial count was recorded on day- 28 following *Clostridium perfringens* challenge. The 0.1% probiotic treatment showed 7.2% increase in average feed intake ($P=0.05$) and 8% increase in body weight compared to T2. In 0.1% treatment body weight was 5% higher than T3 ($p=0.02$). It was also observed that 0.1% treatment had improved feed conversion ratio (1.77) on 6th week. No effect of treatment was observed on mortality and ileal bacterial count. The current study indicated that 0.1 % use of probiotic had positive response in *C. perfringens* challenged broilers.

Keywords: *Bacillus subtilis* PB6, antibiotic growth promoters (AGP), *clostridium perfringens*, CloSTAT, Broilers

S1-0258 The effect of inulin and/or wheat bran supplementation during early life (0–d10) or during the entire rearing period on the performance of broilers

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Fibers could improve the host's health by inducing favorable changes in intestinal microbiota. The complex intestinal composition of the microbiota develops early in life. In our study, the use of inulin and/or wheat bran during only the starter phase or during the entire rearing period was investigated in broiler chickens. 960 one-day old male broilers (Ross 308) were fed four types of diet (4% inulin (IN), 10% wheat bran (WB), 4% inulin+10% wheat bran (WB+IN) or a control diet without inulin and wheat bran(CON)) for 10 days (6 pens per group). Thereafter, 3 pens from the experimental groups continued to use grower and finisher diets with same supplementation, while 3 pens turned on control grower and finisher diets. Body weight (BW) was recorded weekly, feces and ileal digesta were collected at the end of the starter (d10), grower (d23), and finisher period (d39). IN provided during the starter phase had no effect on BW, but IN during the entire period decreased BW compared to control chickens on d14. Adding WB with or without IN only in the starter phase, increased BW compared to the control group on d7, d14, d21, and d28, while this effect was observed only for WB supplemented groups (starter phase) on d35. The use of WB during the entire rearing period still had an increased BW on d14 compared to the control group, but this difference disappeared from d21 onwards. The supplementation of both IN and WB during the entire period could extend the beneficial effect of WB with one week (increased BW also on d21). The relative liver and spleen weight (d10 or d39), feed intake, ileal and total digestibility of energy, NDF, ADF or fat of the starter diet were not significantly affected by the dietary treatment. Furthermore, ileal and total digestibility of the grower and finisher phase, short-chain fatty acids and composition of the gastrointestinal microflora are being determined. In conclusion, the use of WB only during the starter period gave highest BW on d35.

Keywords: broiler, inulin, wheat bran, intestinal performance

S1- 0259 Diets varying in protein content and indigestible protein fractions influence the growth performance of broilers

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Poorly digested protein (high indigestible fraction) has been suggested to result in poor broiler health and performance because of the negative effects of protein fermentation. To test this suggestion, this study evaluated the effects of three dietary protein levels (24, 26 and 28%) with low or high indigestible protein (LIP, HIP) on broiler performance. The trial was completely randomized, with a 2x3x2 factorial arrangement, where dietary protein levels, dietary indigestible protein and gender were the main factors. Ross 308 male (M, 1944) and female (F, 2232) were randomly allocated to 72 litter floor pens and fed one of six diets varying in protein level and indigestible protein fraction (24- LIP, 24- HIP, 26- LIP, 26- HIP, 28- LIP, 28- HIP) from 1-32 d of age. All diets met broiler grower nutrient specifications, contained no medication and were formulated to the same level of digestible methionine. Wheat was the cereal grain in all diets and protein sources in the LIP diets were soybean meal (SBM) and fish meal, while HIP diets used SBM, corn gluten meal, porcine meal and corn distillers dried grains with solubles. Birds were vaccinated with Coccivac-B52 on d 5. Differences were considered significant when $P \leq 0.05$. Body weight at 32 d was affected by protein content (26>28 with 24% intermediate), protein indigestible fraction (LIP>HIP) and gender (M>F). Feed to gain ratio (mortality corrected) for the overall trial was affected by protein level (24>26=28) and protein indigestible fraction (HIP>LIP). Total mortality (%) was affected by gender (M>F) and protein indigestible fraction (LIP>HIP), but not by protein level. Interactions between main effects were not found for the above characteristics. Total feed consumption was affected by gender (M>F) and the interaction of protein level and indigestible protein. In conclusion, dietary protein level and the proportion of indigestible protein can affect performance when broilers are fed nutritionally adequate diets.

Keywords: undigested protein, cocci- vaccination, antibiotic-free, chicken

S1- 0260 Effects of Azadirachta, Spondias and Chromolaena as alternatives to in- feed antibiotics on growth and histopathology of selected organs in broiler chickens

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The effects of *Azadirachta indica* (AI), *Spondias mombin* (SM) and *Chromolaena odorata* (CO) as alternative to in- feed antibiotics on growth and histopathology of broiler chickens were studied. 288 broiler chicks were randomly allocated to six dietary treatments in a Completely Randomized Design and data was analysed using one-way ANOVA. Each treatment had 48 birds which were further divided into four replicates of 12 birds each. Feed and water were provided ad libitum daily for the 57-day feeding trial. Diet 1 was a basal diet without antibiotics nor alternative, diet 2 contained Neoceryl, Diet 3 had 5g milled AI leaves, while Diet 4 had 2.5g each of AI and SM. Diet 5 had 2.5g AI and 2.5g CO and Diet 6 contained 1.7g each of AI, SM and CO per kg diet. Performance of the birds was determined. At day 57, three birds were sacrificed per replicate, liver and lungs were excised for histopathological examination. Birds on diet 2 had the highest final weight (2125g) while birds on diet 4 had the best FCR (2.30). Histopathological change ranged from moderate congestion of hepatic sinusoid, cellular infiltration and hepatocellular necrosis of the liver in all treatments which might be due to antinutritional factors (Tannins) in the test ingredients while treatments 1 and 2 showed normal liver and lungs. Treatment 5 induced necrosis and erosion of epithelia lining of the bronchus and infiltration of lung mononuclear cells. In conclusion, birds appeared healthy at maturity, practitioners should however exercise caution in using the alternatives for prolonged period considering their effects on histology of organs examined.

Keywords: azadirachta indica, spondias mombin, chromolaena odorata, broiler, histopathology

S1- 0261 Response of broiler chickens fed diets containing raw soybean meal and supplemented with varying levels of microbial protease

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A 3 x 2 factorial study was conducted to assess the effects of feeding diets containing three levels of raw soybean meal (RSBM), replacing commercial SBM at 0, 15 or 25 % and supplemented with 0 or 200 mg mono-component protease (Nocardopsis prasina, DSM) per kg diet. Each diet contained 2000 FYT microbial phytase per kg and was replicated six times, with nine birds per replicate. Birds were housed in cages, in climate-controlled rooms and offered a starter (1-10 d), grower (11-24 d) and finisher (25-35 d) corn-soybean based diets, formulated to Aviagen standards for Ross 308. The maximum trypsin inhibitor content of the diets containing RSBM was around 10193.4 TIU/kg. Feed consumption to 10 d ($p=0.08$) and 35 d ($p=0.07$) tended to decline with an increase in RSBM. Body weight gain (BWG) was also reduced in the periods of 1-10 d ($p<0.01$) and 1-24 d ($p<0.05$) but was only marginally reduced (provide a % of the reduction) ($p=0.07$), when measured at 35 d. The BWG and feed efficiency of birds were improved ($p<0.05$) due to supplementation of microbial protease in the period, 1-24 d. The interactions between protease x RSBM were not significant ($P>0.05$) for FI or FCR. The weight of the pancreas as most of the internal organs at 24 d, was increased ($p<0.001$) as a result of inclusion of RSBM. Except for the weight of the bursa, protease supplementation had no effect ($p>0.05$) on other internal organs. In conclusion, RSBM could be included at up to 25 % in broiler chicken diets if the diets are supplemented with the test protease and phytase products.

Keywords: broiler chickens, raw soybean meal, microbial protease, phytase, trypsin inhibitors

S1- 0262 Intestinal mucosal morphometry and digestive function of broiler chickens fed on diets containing raw soybean meal supplemented with microbial enzymes

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A 3x3 factorial study was conducted to assess the impact of feeding diets containing raw soybean meal (RSBM) supplemented with a microbial mono-component protease product. The SBM was replaced by RSBM at 0, 10 or 20 %. Each was replicated 6 times, with 9 birds per replicate. Birds were housed in cages, in climate-controlled rooms and fed on starter and grower diets, formulated to Aviagen standards for Ross 308. The concentration of trypsin inhibitors in the RSBM was 13098.0 TIU/g, prior to mixing and ranged between 1730.5 and 9913.2 TIU/g in the treatment diets. The concentration of protein in the pancreas (at 10 d age) was reduced ($P<0.05$) in response to increasing level of RSBM. Pancreatic tissue protein content was increased ($P0.05$) effect on activities of jejunal enzymes. Because of protease supplementation, pancreatic protein content, and activities of digestive enzymes, including trypsin, chymotrypsin and general proteolytic activity were increased ($P<0.05$) at 24 d of age. The activity of alkaline phosphatase in the jejunum tended ($P=0.05$) to be reduced with increasing level of RSBM, whereas it was improved ($P<0.01$) as a result of supplementation with protease and the interaction effects ($P<0.01$) between RSBM and protease. The interaction between protease and RSBM was significant for villus height ($P<0.05$), mucosal depth ($P<0.01$) and also tended to affect apparent villus surface area ($P = 0.06$) and crypt depth ($P=0.08$) at 10 d of age. However, due to protease supplementation there were protease x RSBM interactions for improved villus height ($P=0.08$) and mucosal depth ($Pp=0.08$) at 24 d of age. Results of this study show that protease supplementation of the test diets improved the activities of some digestive enzymes and mucosal morphometry at the jejunum.

Keywords: raw soybean meal, enzyme activities, mucosal morphometry, trypsin inhibitors, broilers

S1-0263 The effect of *Lactobacillus acidophilus* on cholesterol reduction in laying hens

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The objectives of the present study were to evaluate the effect of probiotic *Lactobacillus Acidophilus* on Cholesterol reduction and its role in layer performance. In the present study, one hundred-eighty-nine 40-wk-old laying hens (Hy-line) were randomly assigned into 3 groups with 3 replicates of 21 birds each (63 laying hens per group), and fed diets supplemented with 0.00, 0.10 and 0.20% *Lactobacillus Acidophilus*. Layer performance, plasma lipid profile of liver & egg yolk cholesterol were evaluated. Egg production was not affected during the 6-wk of bacterial feeding trial, but *Lactobacillus Acidophilus*, at the level of 0.20%, significantly ($P<0.05$) improved egg weight and feed consumption, and significantly reduced the feed conversion ratio at the levels of 0.10 and 0.20%. Even though *Lactobacillus Acidophilus* at the levels of 0.10 and 0.20% significantly decreased the plasma LDL-cholesterol by 10.9 and 16%, respectively and increased plasma HDL-cholesterol by 9.6 and 10.6%, respectively, compared with control, the changes in plasma total cholesterol concentration were not significant. Furthermore, the results showed that the cholesterol concentrations of liver and egg yolk were reduced significantly ($P<0.05$) when compared to control group for hens fed diets supplemented with 0.10 and 0.20% *Lactobacillus Acidophilus*. In conclusion, *Lactobacillus Acidophilus* showed cholesterol reduction effect in plasma and liver. Therefore, *Lactobacillus Acidophilus* may be a good candidate for commercial production of low cholesterol eggs along with positive impacts on hen-laying performance.

Keywords: laying hens, *Lactobacillus Acidophilus*, cholesterol, production performance

S1-0264 Effect of heat treatment on true metabolizable energy (TMEn) and amino acid digestibility of expeller-extracted soybean meal

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Soybean meal has been used as a major protein source in poultry and livestock diets. Expeller-extracted soybean meal (ESBM) is the product that remains after removing the oil from whole soybeans using expeller technology. However, the expeller process, which includes extrusion, does not produce enough heat for sufficient time to inactivate the majority of the trypsin inhibitors present in ESBM. Therefore, ESBM contains higher trypsin inhibitor levels than conventionally processed solvent extracted SBM (SSBM). The present study was conducted to evaluate the effect of autoclaving ESBM on urease activity, protein dispersibility index (PDI), KOH solubility, trypsin inhibitor level, true amino acid digestibility, and true metabolizable energy (TMEn). Soybean cake was obtained from a local plant that uses the extrusion-expeller technology and was ground in a roller mill to produce ground ESBM. After grinding, ESBM was divided into two portions. One half of the ESBM was autoclaved at 100°C for 30 min. The average particle size of the ground ESBM was 1,290 µm. Autoclaving reduced the trypsin inhibitor levels from 21 TIU/g to 6 TIU/g, urease activity from 0.28 to 0.05 Δ pH, PDI from 18 to 9%, and KOH solubility from 85 to 59%. Subsequently, ESBM (autoclaved and as received) was fed to cecectomized roosters to measure TMEn and true amino acid digestibility using the total excreta collection method. Autoclaving ESBM did not have an effect on true amino acid digestibility ($P>0.05$). However, autoclaving ESBM increased TMEn from 2,867 kcal/kg to 3,418 kcal/kg ($P<0.01$). The improvement in TMEn (21%) suggested that ESBM would normally require additional heat treatment to improve its nutritional value.

Keywords: expeller, soybean meal, heat treatment, cecectomized roosters, digestibility

S1-0265 Effect of different form of lab, isolate from virgin coconut oil processing waste on performance and meat quality of broilers

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Giving of lactic acid bacteria, the isolate from the Virgin Coconut Oil processing waste in liquid form direct by oral have been demonstrated to improve broiler performance and decreasing cholesterol of meat and cholesterol of blood, however the oral administration is not effective at the farmer level. From previous studies it is reported that using corn for inoculum media and encapsulation using skim milk can maintain the ability and stability of LAB up to 10 weeks. The aim of this experiment was to evaluate the effect of different form LAB i.e: Without probiotics (WP), Liquid probiotics (LP), Probiotic corn Inoculum 1x (PC1), Probiotic corn Inoculum 2x (PC2), Probiotic encapsulation 1x (PE1), Probiotic encapsulation 2x (PE2), (1x dose equal $\pm 1.3 \times 10^8$ cfu/g) on performance and meat quality of broilers. This study used 144 day old broiler chicks (Cobb). Chickens were divided into 6 treatments and 4 replications. The treatment started at eight days age, administered every two weeks until 42 days age. The ration contain 21% protein and Energy 3080 kcal ME/kg. Data were subjected to ANOVA the proc Mixed procedure of SAS. The results showed that the giving of probiotic no significant effect ($P > 0.05$) on feed intake, however leading the number of LAB in small intestine, body weight and carcass significantly ($P < 0.01$). The administration of probiotic caused decreasing the feed conversion, abdominal fat and the content of fat and cholesterol of broiler meat significantly ($P < 0.01$) compared WP. Differences form of probiotics, significantly ($P < 0.05$) affect the body weight, feed conversion, carcass percentage and fat content of meat, but did not affect meat cholesterol content. It can be concluded that the probiotic using corn as inoculum 2x (PC2) indicated better performance with the fat and cholesterol meat content were lower.

Keywords: lactic acid bacteria, inoculum, encapsulation, performance, broiler

S1-0266 Comparative evaluation of different oils in broiler ration

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The growth performance of broiler chicken due to dietary inclusion of combinations of different commonly available oils, viz., coconut oil, fish oil, groundnut oil and rice bran oil were studied by rearing two hundred, sex separated, day-old, commercial (Vencobb) broiler chicks belonging to single hatch in deep litter system. All the chicks were wing banded, weighed and randomly allotted into five treatment groups with five replicates of eight chicks each for a period of six weeks. The treatment groups consisted of T1 (Basal diet with Fish oil), T2 (Basal diet with Fish oil + Coconut oil), T3 (Basal diet with Fish oil + Groundnut oil), T4 (Basal diet with Fish oil + Rice bran oil), T5 (Basal diet with Coconut oil + Groundnut oil + Rice bran oil). The experimental feed was formulated according to the Vencobb standards for different treatment groups. All the diets were isocaloric and isonitrogenous. The oils viz., coconut oil, fish oil, groundnut oil and rice bran oil were included in the ration at 1 per cent, 2 per cent and 3 per cent level in pre-starter, starter and finisher diets, respectively. The analysis of variance of data on mean body weight (g), body weight gain (g) and feed conversion ratio of broilers revealed no significant difference between treatment groups from first to sixth week. Groundnut oil combined with fish oil (T3) in the basal diet recorded numerically higher body weight gain than all other combination of oils fed group. Different oils and their combinations with fish oil did not show any significant influence on carcass characteristics and sensory evaluation of meat in broilers. Based upon this study, it is concluded that broiler mash formulated combination of coconut oil, groundnut oil and rice bran oil in the ration at 1 per cent, 2 per cent and 3 per cent level in pre-starter, starter and finisher diets, respectively might increase the production performance and thereby better profit margin in broiler production.

Keywords: different oils, broiler ration, growth performance

S1-0268 Long term effects of dietary microalgae on productive performance of laying hens and egg docosa-hexaenoic acid concentration

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A study was conducted to investigate the long term effects of dietary supplementation of dehydrated whole cell microalgae (All- G- Rich, Alltech Inc.) on productive performance, egg-shell quality and egg fatty acid composition of laying hens. Day- old Hy- Line Brown pullets were fed a corn-soybean meal control diet alone or supplemented with 1 or 2% All-G-Rich. The birds were raised in pullet cages until 16 wk of age. Then seven groups of 12 pullets for each of the three treatments were transferred to layer cages using two birds per cage (25 x 41 cm). Pullets were photo stimulated with 16L: 8D and dietary treatments continued through 70 wk of production. Dietary supplementation of All-G-Rich decreased ($P<0.05$) overall feed intake (114, 112 and 110 g/hen/d) and increased ($P<0.05$) body weight (2.03, 2.09 and 2.11 kg) at 40 wk of production. No effects of treatments on egg production (average = 84%), egg weight (average = 61.5 g), and feed conversion ratio (average = 1.59 kg/dz) were detected. Per cent eggshell (average = 9.9%) and eggshell breaking strength (average = 2.76 kg force) were not affected by dietary supplementation of All-G-Rich. The docosa-hexaenoic acid (DHA) content in egg was linearly increased ($P<0.01$) with increasing levels of dietary All-G-Rich. The average egg DHA values were 85, 187 and 240 mg/100 g of egg for hens fed 0, 1 and 2% All- G- Rich, respectively. The redness of egg yolk was increased ($P<0.05$) by dietary All-G-Rich. The results indicate that supplementing All-G-Rich in layer diet can enrich DHA in eggs and increase redness of yolk color with no effect on productive performance of layers.

Keywords: layer, microalgae, DHA, egg, performance

S1-0269 Antioxidant effect of different dietary levels of DL- methionine and its hydroxyl analog in young turkeys

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The aim of the study was to verify the hypothesis that dietary levels of DL-methionine (DL-Met) and its hydroxyl analog (MHA) can improve the antioxidant and immunological protection of young turkeys. A total of 512 one-day-old female Hybrid Converter turkeys were randomly divided into 4 groups, with 8 replicates per group and 16 birds per replicate. All birds were fed identical wheat-soybean meal-based diets, supplemented with DL-Met or MHA. In the first and second 4-week periods of feeding, the crude protein content of diets was 27.1% and 24.9%, respectively, and the final dietary inclusion levels of methionine were consistent with NRC (1994) recommendations (0.55% and 0.35%, respectively) or 40% higher (0.77% and 0.50%, respectively). At 56 days of age, eight birds representing the average body weight of each replicate were selected to collect blood samples and determine their biochemical parameters, antioxidant and immune status. An increase in dietary Met concentrations, regardless of Met source, increased the body weight gains of turkey and it had no effect on feed efficiency ratio. No differences were found in PGx activity in the blood, whereas SOD activity increased in response to higher dietary levels of Met. DL-Met increased SOD activity, as compared with diets containing MHA. In comparison with DL-Met, MHA decreased catalase activity and glutathione concentrations, and it increased plasma lipid peroxide levels. Higher dietary Met content increased total antioxidant capacity measured as the ferric-reducing ability of plasma. At lower Met levels, DL- Met exerted a stronger antioxidant effect than MHA. The level and source of Met had an insignificant influence on the redox status of the small intestine and liver of turkeys. It can be concluded that increased dietary levels of Met improved antioxidant protection in young turkeys; the observed effect depended on the source of Met and it was more pronounced when diets were supplemented with DL-Met than MHA.

Keywords: turkeys, DL-methionine, MHA, antioxidant activity, growth performance

S1-0270 Faba beans (*Vicia faba* L.) with a different tannin content as a substitute for soybean meal in the diet of growing-finishing turkeys

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This study evaluated the effects of dietary replacement of soybean meal (SBM) with graded levels of high- and low- tannin faba bean (FB) seeds on growth performance and meat quality in finishing turkeys (13-18 weeks of age). Male Hybrid Converter turkeys were divided into 7 groups (7 different finisher diets): a control diet, which contained SBM, and experimental diets where SBM was replaced with FB at 10%, 20% and 30% of high-tannin (HT) seeds (HT10, HT20, HT30, respectively) and of low-tannin (LT) seeds (LT10, LT20, LT30, respectively). In comparison with HT seeds, LT seeds did not affect feed intake, body weight gains (BWG) or the mortality rates of turkeys, but they significantly improved FCR. The meat of turkeys fed diets supplemented with HT faba bean seeds was darker, with a lower contribution of yellowness, and it had a lower content of dry matter and crude ash than the meat of turkeys fed diets containing LT faba bean seeds. Diets with HT and LT seeds changed the proportions of a few fatty acids in turkey meat, and the most important indicators of meat quality, such as the concentrations of n-6 PUFAs, n-3 PUFAs and the n-6/n-3 PUFA ratio, were not influenced by the tannin content of FB seeds. Regardless of seed variety, dietary inclusion of FB up to 30% instead of SBM, had no adverse effect on the performance parameters or carcass traits of turkeys, including proximate composition, physicochemical properties and the intensity of the analyzed attributes of meat taste, aroma and texture. In conclusion, both low- and high-tannin faba bean seeds can be added to finisher diets for turkeys at up to 30% as an effective substitute for soybean meal without compromising the key variables of performance and with no negative effects on carcass traits or meat quality. In comparison with HT seeds, LT seeds improved feed utilization and selected parameters of the nutritional quality of turkey meat, with simultaneous deterioration in selected parameters of meat color.

Keywords: Faba bean, turkey nutrition, growth performance, meat quality

S1-0271 Effects of dietary tryptophan supplementation on performance, antioxidative property, immunity and mRNA expression of amino acid transporter in ileum mucus in Chinese yellow-feathered broiler breeders

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The effects of dietary Trp levels on performance and biochemical parameters of plasma and intestinal mucus in broiler breeder hens was investigated in this study. A total of 780 Lingnan yellow-feathered broiler breeder hens were randomly divided into 1 of 5 dietary treatments with 6 replicates per treatment (26 birds per replicate). The breeder hens were fed either the basal diet or the basal diet supplemented with 0.04%, 0.08%, 0.12% and 0.16% Trp from 29 wk to 38 wk. The results showed that dietary supplemental 0.08% and 0.12% Trp increased laying rate by 7.67%, 6.36%, respectively ($P < 0.05$). The content of Trp of egg was not affected by dietary Trp supplementation ($P > 0.05$). An increase of fertilization rate of total eggs was observed in breeders fed 0.16% Trp, and hatchability was higher in breeders fed 0.12% and 0.16% Trp than those of 0.08% Trp. Dominant follicle weight was increased by supplemental 0.12% and 0.16% Trp ($P < 0.05$). The content of glutathione (GSH) and GSH to oxidized GSH ratio of plasma were reduced by 0.04%, 0.08% and 0.16% Trp addition. A higher activity of glutathione S-transferase of plasma was observed in 0.15% Trp treatment compared to 0.12% and 0.16% Trp treatment ($P < 0.05$). The activity of Na^+/K^+ -ATP of plasma in birds fed 0.16% Trp was lower than those fed 0.15% Trp and the control birds ($P < 0.05$). There were significant up-regulations of dietary Trp supplementation on ribosomal S6 kinase, neutral amino acid transporter (B0AT1), nuclear factor erythroid-2 related factor 2, toll-like receptor 4, tumor necrosis factor- α , interleukin-6 transcripts of ileal mucus ($P < 0.05$). The gene expressions of mammalian target of rapamycin, aminopeptidase, peptide transporter 1 were not influenced by dietary Trp level ($P > 0.05$). It concluded that dietary Trp supplementation significantly improved reproductive performance, antioxidative property, and neutral amino acid transportation in ileum mucus of broiler breeder hens.

Keywords: broiler breeders, tryptophan, reproduction performance, dominant follicle, neutral amino acid transporter

S1-0273 Biological evaluation of azolla in combination with carbohydrases in poultry broiler bird

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A study was under taken to explore the nutritive value of Azolla pinnata as unconventional feed ingredient in poultry broiler feeding. An experiment was conducted on 88, day old chicks, which were divided into 4 main groups (2 control + 2 treatments) with in replicates having 11 chicks in each replicate. The ration was formulated to have standard control diet T0 and T1 with and without carbohydrase and phytase enzymes and test diets T2 and T3 based on 5 per cent substitution with dry Azolla with and without carbohydrase and phytase enzyme as an ingredient compared to a standard formulated conventional diet in a way that the crude protein and metabolizable energy values were similar in all treatments (T2, T3) and control (T0, T1). Overall biological growth performance of the Azolla fed groups (T2,T3) was found to be comparable to the birds offered standard soya flake formulated feed (T0, T1). Growth performance did not exhibit any significant ($P < 0.05$) difference amongst various treatments whereas the feed intake per unit gain in live weight was found to be significantly ($P < 0.05$) higher for the Azolla fed groups exhibiting poor feed conversion efficiency. Azolla substitution seems to improve the palatability of the feed as indicated by higher feed intake but the supplementation of carbohydrase enzyme did not affect growth and feed conversion efficiency. Results obtained revealed higher availability of the minerals Ca and P in Azolla fed groups as exhibited by higher deposition of the minerals viz. Ca and P in the storage organ viz. tibial bone. Overall it is thus concluded that azolla can be safely incorporated at 5 per cent level in poultry broiler feed.

Keywords: azolla, poultry, broiler, nutrients, growth, FCR, enzymes, phytase

S1- 0275 Cost reduction in broilers through low protein diets

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Abstract (for oral presentation) Title : Cost reduction in broilers through low protein diets D.J. Torne, R.N. Bade and S. V. Deshmukh Dr. B. V. Rao Institute of Poultry Management and Technology, India. In an attempt to reduce cost of feed in broiler production, a feeding trial was conducted for 42 days in open house broiler sheds with deep litter. VenCobb400 day old 4000 commercial broiler chicks distributed in two groups with 16 Replicate of 125 chicks were fed diets formulated with approximately 0.5 per cent lower CP (LCP) than the standard protein (SCP) diets to the same required ME balanced with appropriate amino acid profile. The data was statistically analyzed by completely randomized design. The diets had CP% 22.76& 22.27; 20.49&20.00; 18.85&18.34; 18.05& 17.57 in prestarter, starter, grower & finisher respectively with 2952,3071,3175, 3204Kcal ME/Kg with all amino acids in adequate quantity for normal growth of broilers. The ratio of lysine to CP in different diets ranged between 5.43 to 5.64 with the ratio of lysine to all essential amino acids in diets was within normal range with similar ME to lysine ratio of 0.31 to 0.41. The performance of birds was at par statistically indicating no adverse effect due to reduction in CP content at any stage of growth. The final bodyweights and feed intake per bird in SCP and LCP groups was 2.243&2.252 Kg; 4.06 & 4.05 Kg with FCR 1.81 and 1.80 and meat yield of 75.00 and 75.12 % respectively. Profit per bird was more (INR18.75) for LCP group than the control (INR16.35). The extra profit of treatment groups was mainly attributed to lesser feeding cost and higher live body weights. From study it was concluded that reduction in crude protein content in the diet of commercial broilers with balanced amino acid profile increased profitability without any adverse effect on the performance.

Keywords: low protein diet, cost reduction, broilers

S1-0276 The effect of six dietary fats on tissue fatty acid composition and growth parameters of broiler chickens

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Adding flaxseed oil to chicken diets has been shown to significantly increase the n-3 PUFA (including long chain n-3) content in chicken meat and in some experiments has significantly improved the growth rate (GR) and reduced the feed conversion ratio (FCR) of the birds. It is not clear whether this latter effect is related to differences in the types of fatty acids present (e.g. n-3 polyunsaturates vs monounsaturates and saturates), or other reasons. To address this we fed Cobb 500 male chickens (n=480) one of 6 different diets containing 4% (w/w) flaxseed (high n-3), corn (high n-6), macadamia (high n-7), canola (high n-9), coconut (high saturates) oils or beef tallow (control). Growth and feed intake were measured each week. After 6 weeks, tissues including blood, adipose, breast and leg meat, liver, heart and brain, were analysed for crude fat content and fatty acid composition. All the birds grew well, and there were no significant differences in GR and FCR among dietary groups. The crude fat content of the different tissues were similar across dietary treatments, however, there was a substantial effect of diet on the fatty acid composition of most tissues. There were strong positive correlations between the diets' fatty acid composition and those of the blood, adipose tissue, breast and leg meat and weaker correlations for the liver and heart. The fatty acid composition of the brain, with the exception of n-3, was unrelated to dietary fatty acid composition. These results show that, at the levels used in this trial, n-3 deposition in most tissues is related to the chemical composition of the fat in the diet, but not its physical state (liquid/solid) or origin (plant/animal). This supports our hypothesis of the direct positive effect of n-3 intake on n-3 levels in the tissues particularly in the edible organs.

Keywords: fatty acids, broilers tissues, diet

S1-0277 Effects of diet inclusion of multi-activity enzymes enriched in xylanase and arabinofuranosidase on laying hens performances fed wheat-based diets with different nutritional densities

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A new carbohydrase has been developed with enlarged xylanase and arabinofuranosidase activities, increased total activity and enhanced number of protein enzymes. The experiment was designed to evaluate the efficacy of such an enzyme (Rovabio® Advance P, Adv) in wheat-fed layers on production performance and egg quality. One thousand nine hundred and twenty hens were allocated into six treatments as follows: a positive control (PC) diet formulated to be adequate in nutrient and two negative control (NC) diets similar to PC but with a lower metabolisable energy and digestible amino acids levels (-1.5% for NC1 and -3% for NC2) with and without enzymes (Adv). These six treatments were divided into 16 replicates of 20 Leghorn laying hens. Laying performances and egg quality traits were recorded between 46 to 62 weeks of age. For the overall period, results showed that the reformulation (NC1, NC2) reduced egg weight and impaired feed intake (FI) and feed conversion ratio (FCR) compared to the PC ($P < 0.001$). No impact of nutritional reduction neither on egg quality (Fracture force, Haugh Units and Static Stiffness) nor on final body weight (BW) were shown. Addition of the enriched xylanase and arabinofuranosidase enzyme complex significantly improved egg weight, egg mass, FCR and final BW. The Adv enzyme addition on reformulated diets (NC1+Adv, NC2+Adv) promoted similar laying performances to those observed for PC without enzymes. Concerning egg quality, enzyme addition enhanced the static stiffness and Haugh Units compared to non-added diets. These data confirm that enriching NSPase in xylanase and arabinofuranosidase activities improved laying performances and egg quality. These improvements might suggest that laying hens were able to make a better use of dietary nutrients from feed ingestion.

Keywords: NSPase, diet reformulation, laying hens, performances, egg quality

S1-0278 Effect of protease on growth performance, intestinal morphology, gut barrier function, and gut microbiota of broilers

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Two studies were conducted to evaluate effect of a protease (CIBENZA® DP100, Novus International, Inc.) on growth performance, intestinal morphology, gut barrier function, and gut microbiota of broilers. Trial 1 consisted of 5 treatments: positive control (PC), negative control (NC), NC+protease, NC+BMD, and NC+protease+BMD. Compared to PC diets, crude protein, amino acid, and ME levels in NC diets were reduced by about 2.1%, 2.7%, and 17 kcal/kg respectively. Each diet was fed to 8 replicate pens of 22 male broilers. Body weight, feed intake, FCR, and mortality were determined at d 19 and 36. On d 13, Gut morphometry and concentration of serum endotoxin were measured. Trial 2 consisted of 2 treatments, NC and NC+protease, with 4 replicate cages of 8 birds. On d 24, excreta samples were collected, DNA extracted, and subjected to bacterial community analysis by sequencing of 16S rRNA gene. Data were analyzed by ANOVA, and means were separated by Fisher's LSD test. Orthogonal contrasts were made to test effect of protease or BMD in trial 1. A P-value of ≤ 0.05 was considered significant. Adding protease or BMD alone did not affect 0-19 d FCR, but adding both together significantly improved it suggesting protease and BMD could complement each other. The NC birds had higher 0-36 d FCR than the PC birds; protease, BMD, or their combination improved FCR to a level comparable to PC birds. Contrast test indicated that BMD reduced mortality, duodenal crypt depth, and serum endotoxin concentration. Ileal gut muscle width of broilers was increased by BMD. In trial 2, protease decreased the proportion of firmicutes phylum and increased species evenness and diversity index. These trials demonstrated that both protease and BMD were effective in restoring the increased FCR from lower dietary protein and ME in broilers, and were able to improve gut health of broilers. While their modes of action were different, additional benefits could be observed when they were used together.

Keywords: broiler, protease, bacitracin methylene disalicylate, microbiota

S1-0279 Benefits of protected benzoic acid in broilers subject to two different dietary challenges

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One study was conducted to evaluate effect of protected benzoic acid (PBA, Provenia®, Novus International, Inc.) on growth performance and gut health of broilers fed diets either high in viscosity (HV) or medium in viscosity but high in protein (MVHP) in comparison to bacitracin methylene disalicylate (BMD). The study consisted of 6 treatments arranged in a 2 x 3 factorial design, with 2 diet types (HV and MVHP) and 3 additives (none, PBA providing 240 g/MT benzoic acid, and BMD providing 110 g/MT bacitracin). The HV diet was rye wheat and SBM based; rye level was reduced but poultry meal was included to reach 30% CP in MVHP diet. Each treatment had 8 replicate cages of 9 Ross 708 male broilers. Performance parameters including BW, feed intake, FCR, and mortality were determined on d 7, 14, 21, and 27. Ileal *Clostridium perfringens* (Cp), serum glutathione peroxidase activity (GPx), serum uric acid concentration, and digesta viscosity were measured on d 15, 16, 22, and 28 respectively. Data were analyzed by two-way ANOVA with pen as experimental unit, and means were separated by Fisher's LSD test. A P-value of ≤ 0.05 was considered significant. Birds fed HV diets had lower body weight and worse FCR than those fed MVHP diets despite similar feed intake. Digesta viscosity was higher, and serum uric acid concentration was lower in HV birds. Protected benzoic acid consistently improved FCR of broilers throughout the trial regardless of diet type, and the effect was comparable to BMD. The PBA or BMD beneficial effect on FCR was more pronounced from 0-14 d for MVHP diet, but from 14-27 d for HV diet. Both PBA and BMD unexpectedly increased ileal Cp irrespective of diet type. Serum GPx activity was also increased by BMD in both diets. In Summary, both HV and MVHP models can be used to test gut health benefits of certain feed additives in broilers; protected benzoic acid delivered similar benefits to BMD in terms of improving growth performance and modifying gut microflora.

Keywords: broiler, benzoic acid, bacitracin methylene disalicylate, gut health model

S1-0280 Non-phytate phosphorous requirement of Feather-frizzled broilers aged from 1 to 21 days

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There is little known about the requirements of Feather-frizzled broilers in southern areas of China. An experiment was conducted to determine the non-phytate phosphorus requirement for Feather-frizzled broilers aged from 1 to 21 days by using broken line model. A total of 360, 1-day-old broilers were assigned to 1 of 6 treatments with 6 cage replicates of 10 birds each using a completely randomized design and were fed a corn-soybean meal based diet (1.02% Ca) containing 0.13% (control), 0.23%, 0.33%, 0.43%, 0.53% and 0.63% non-phytate phosphorous for 21 days. Feed consumption was recorded daily, whereas body weight was obtained at the start and at the end of the trial. At the end of the trial, one chick from each replicate was chosen to collect the right tibia and the middle toe for analyses of tibia quality and toe characteristics. According to the fitted broken line models of the following responses to different dietary non-phytate phosphorus, the optimal non-phytate phosphorus requirements of broilers for maximum ADG, ADFI, feed efficiency, tibia strength, dry weight of defatted tibia, tibia ash content and P content in tibia ash, dry weight of defatted toe, toe ash weight, toe ash content, P content in toe ash were 0.25%, 0.26%, 0.36%, 0.39%, 0.37%, 0.37%, 0.24%, 0.36%, 0.37%, 0.27% and 0.23%, respectively. Results showed that the range of optimal non-phytate phosphorus requirement of Feather-frizzled broilers fed a corn-soybean meal basal diet from 1 to 21 days of age was from 0.23% to 0.39%.

Keywords: Feather-frizzled broilers, phosphorus, tibia quality, toe characteristics

S1-0281 Effect of dietary tea seed meal supplementation on performance, carcass and immune function in broilers

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Tea seed meal is the residue of *Camellia oleifera* Abel seeds after camellia oil extraction. The present study aimed to investigate the effect of dietary tea seed meal supplementation on performance, carcass, and immune function in broilers. A total of 150 one-day-old health male chicks were randomly allocated into five dietary treatments. Each treatment had five replicates and each replicate had six chicks. The chicks from control group were fed corn-soybean diet and these from treatment groups were fed corn-soybean diet supplementation with 0.50%, 0.75% and 1.00% tea seed meal. The chicks from positive control group were fed corn-soybean diet supplementation with 300 mg/kg chlortetracycline. The trial period was 35 d. The performance, carcass and immune index of broilers at 21 d and 35 d were measured. The results showed that the broilers fed with 0.50% tea seed meal, mortality rate was significantly decreasing at 8.67% ($P < 0.05$) at 14 d compared with control group. The performance and carcass were no significant difference between 0.50% tea seed meal group and control group ($P > 0.05$). With the dietary tea seed meal supplementation increasing, the average daily gain (ADG) and feed intake (FI) of broilers were decreasing ($P < 0.05$ or $P < 0.01$). In 0.50% tea seed meal group, the IgG content in duodenal mucosa was significantly increasing ($P < 0.05$) at 21 d compared with control and chlortetracycline groups and TNF- α content was significantly decreasing ($P < 0.01$) at 35 d; the contents of IL-2, TNF- α and SIgA were decreased significantly ($P < 0.05$ or $P < 0.01$), but no significant difference was found between 0.50% tea seed meal and chlortetracycline group ($P > 0.05$). Dietary supplementation with 0.50% tea seed meal could be decreased the mortality rate of chicks and have no poison on performance, carcass and immune function in broilers.

Keywords: broilers, performance, carcass, immune function

S1-0282 Effect of dietary vitamin E levels on growth performance and immune organ development in broilers

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The present study aimed to investigate the effect of dietary vitamin E levels on growth performance and development of immune organs in broilers. A total of 160 one-day-old health female chicks were randomly allocated into five dietary treatments. Each treatment had four replicates and each replicate had eight chicks. The chicks from the five treatments were fed 0, 25, 50, 75 and 100 mg/kg dietary DL- α -tocopheryl acetate, respectively in 42-day trial period. The results were shown that growth performance were tended to be increased with the dietary supplement with different levels vitamin E. Average daily gain (ADG) of chickens were increased at 4.23% and 2.90% ($P > 0.05$) added with 50 mg/kg vitamin E in 22-42 d and 0-42 d, respectively; average daily feed intake (ADFI) of chickens were increased at 7.24% and 7.44% ($P > 0.05$) added with 75 mg/kg vitamin E in 22-42 d and 0-42 d, respectively; feed to gain ratio (F:G) of chickens were decreased at 7.78 % and 7.58 % ($P > 0.05$) with 75 mg/kg vitamin E in 0-21 d and 0-42 d; there were no significant effects for the immune organs weight and index supplement with different levels vitamin E at third weeks ($P > 0.05$); the thymus weight and thymus index were increased with 25 and 100 mg/kg vitamin E in sixth weight ($P < 0.05$ and $P > 0.05$, respectively). The growth performance of broilers were tended to be increased and the development of thymus can be improved.

Keywords: broiler, vitamin E, growth performance, immune organs index

S1-0283 Probiotic and antibiotic affects cecum microbes of broiler as revealed by metagenomics and culture-based techniques

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The microbial communities of cecum of broiler chickens from antibiotic and probiotic supplementation were investigated using conventional culture techniques as well as sequencing of 16S rRNA genes. Day old 144 broiler chicks were fed standard broiler ration with antibiotic/ probiotic. Experimental diets viz: D1: standard broiler ration (Control), D2: standard broiler ration (antibiotic @50 gm/ton in feed), D3: standard broiler ration (probiotic @ 1 gm/2L water) were provided continuously upto 15 days experimental period to compare caecal microbiome using metagenomics and culture dependent assays. Data on body weight, feed intake and mortality were recorded. Caecal samples of 15 day old birds were analysed by 16S rRNA and culture dependent protocols for bacterial isolation (Total aerobic, gram positive, negative and lactobacillus counts). Results, showed that body weight at second weeks were 380.11, 347.41 & 413.1 gms in control, antibiotic & probiotic fed groups respectively. Probiotic fed group exhibited superior FCR (0.99) than control (1.06). No significant difference were found for mean Log₁₀ CFU/g caecal contents on different bacterial growth media for aerobic, lactobacillus, gram positive & negative bacterial counts in different groups, whereas results of sequencing revealed difference in bacterial percent among the groups. Taxonomic distribution of data showed 11 phyla, 21 classes, 34 orders, 68 families and 138 genus. Significantly higher ($P < 0.01$) beneficial bacterial counts (Lactobacillus: 17.55 ± 11.36 and Bacteroides : 11.55 ± 11.62) were found in probiotic than antibiotic fed group. Results of the study indicated that supplementation of probiotic increased beneficial bacteria in the broiler caeca however; the same were decreased with antibiotic supplementation.

Keywords: metagenomics, culture dependent, sequencing, taxonomic distribution

S1-0284 Role of exogenous enzymes and insoluble non-starch polysaccharides supplementation in commercial broiler diets

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In a thirty-five day feeding trial, the role of exogenous enzymes and insoluble non-starch polysaccharides (iNSP) supplementation in commercial broiler diets were investigated on performance, carcass weight, and digestive organs weight and length of broiler chickens. The exogenous enzymes used were phytase (1250FTU/kg) and cellulase (250unit/kg), whereas for an iNSP source we used rice hull (RH) at level of 40g/kg. One hundred one- day- old Lohman male broiler chicks were divided into 20 groups of 5 birds each and assigned to the 4 treatment diets in a completely randomized designed. The 4 dietary treatments were: 1) Commercial diets (C as a control diet), 2) CRH (Commercial diets+40g/kg RH) + phytase1250FTU/kg, 3) CRH + cellulase250unit/kg, 4) CRH + phytase1250FTU/kg+ cellulase250unit/kg. Gain (BWG) and feed intake (FI) were recorded weekly, whereas weight and length of GIT and carcass weight were determined at 35d. For performance, differences were found on BWG and FI at week 2 and feed efficiency at week 1 and week 3. Supplementation of phytase and phytase+cellulase in the CRH diets resulted in increasing BWG and FI ($P<0.05$). Feed efficiency of birds fed the CRH diets+cellulase was lower than those fed the other diets ($P<0.05$). The CRH diets supplemented with the exogenous enzymes caused lower duodenal digesta content, shorter jejunum, and lighter jejunal weight than the C diets ($P<0.05$). In addition to that, it tended to increase the percentage of carcass weight around 3% higher than the C diets ($P=0.09$). The result revealed that supplementation of phytase at 1250 FTU/kg and 40g/kg of iNSP in the commercial diets affected broiler performance at the first 3 weeks of age, but increasing percentage of carcass weight and reduced the weight and length of jejunum at 35d of age.

Keywords: exogenous enzyme, insoluble non-starch polysaccharide, broiler performance, carcass weight

S1-0285 Using durian (*duriozibethinus*) waste to support living local chicken in Indonesia villages

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Indonesia has a big population rating at 10 big populations in the world which need a lot of protein sources. There is many protein found in animals body such as chicken. Recently Indonesia people consume a broiler chicken but these sources not sufficient. Common things happen, local chicken is easy to take care. Local chicken eat varieties of waste food. Different from broiler, local chicken can use low quality feed ingredient such as Durian (*Durio zibethinus*) waste, i.e. seed while previous research showed that broiler which fed by Durian (*Durio zibethinus*) seed became stunt. An intensive broiler production need a great grain demand especially corn. Indonesia have increased corn demand from outside substantially, for example, in 2016, Indonesia will import 2.4 billion ton corn. World corn prize rose fluctuates and the effect tends to increase feed costs and broiler price which is in Indonesia causes problem, i.e. inflation. The effort to reduce corn is needed. People start by having more local chicken is one way to cover up the problem. 60% of Indonesia people live in villages where they could rise local chicken intensively and use Durian (*Durio zibethinus*) waste as a corn substitute for chicken feed. Research on utilization Durian (*Durio zibethinus*) seed on local chicken showed that Durian (*Durio zibethinus*) seed could substitute corn up to 15% and Durian (*Durio zibethinus*) seed tends to improve meat quality, i.e. protein content, cooking loss and tenderness.

Keywords: durian (*Durio zibethinus*) waste/seed, corn substitute, meat quality

S1- 0286 The effects of antibiotic, probiotic, oregano, chamomile and ajowan on performance, serum biochemical parameters, microbial culture and Histomorphometry in broiler chickens

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Ban of antibiotics growth promoter (AGP) leading to shock in the poultry industry. Diseases and reduced growth rate were Included among these problems. The purpose of this study was Compare probiotic and several medicinal plants were compared with antibiotic. The experiment performed with 6 treatments and 4 replicates in each treatment (25 birds/replication) in a completely randomized design. Experimental treatments were included: 1) control (basal diet), 2) 2.5 mg/kg Avilamycin antibiotic + Basal diet 3) 0.1 g/kg Protexin probiotic +basal diet 4) 200mg/kg oregano oils + basal diet 5) 200mg/kg chamomile oil + basal diet 6) 200mg/kg Ajwain oil + basal diet. The results of Feed intake, weight gain and feed conversion ratio in the different treatments showed no significant difference ($P > 0.05$). Also, Evaluation of serum Biochemical showed no significant difference in the different treatments ($P > 0.05$). Assessment of microbial enumeration showed significant difference in the number of total aerobic bacteria and *E. coli* ($P < 0.05$). The minimum number of *E-coli* bacteria was found in oregano. The lowest aerobic bacteria were observed in treatments of chamomile, oregano and probiotic. The results of morphology showed significant differences in different parts of the intestine (Duodenum, jejunum, ileum) ($P < 0.05$). In conclusion, the result of this study showed that addition of ajwain seem have a positive effect on Histomorphometry and microbial population also no different effect on performance and serum biochemical compound compare with antibiotic. Therefore, it could be considered as an antibiotic growth promoter replacement for broiler chicks.

Keywords: avilamycin, protexin, performance, medical plants

S1-0289 Influence of oregano essential oil on histological parameters of the jejunal mucosa of broiler chickens

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After the prohibition of the use of antibiotics as growth promoters, some essential oils were used in broiler feed to improve growth performance and digestive tract. In this context, we propose to study the effect of the essential oil of oregano as an alternative to antibiotic growth promoters on histological changes in jejunum of broilers. A total of 36 1-day-old broilers (Arbor Acres) were divided into 2 experimental groups, the first group served as control, received a basal diet based on corn and the second group received the same diet, supplemented with oregano essential oil via their drinking water at an inclusion rate of 150 ml per 1,000 liters, from days 1 to slaughter at day 42. At d 15, 28 and 42, 6 birds from each experimental group were euthanized and the digestive tract was removed. The Morphometry of the median segment of the small intestine (jejunum) was determined by an histological method (micro dissection) using light microscope and software for image analysis. It was observed that oregano essential oil supplementation increased the intestinal absorption area by promoting villus growth in height, width, perimeter and area throughout the trial experiment. However, these differences were statistically non-significant. On the other hand, at 15 and 28 days of age, a significant increase in crypt area ($P < 0.05$) and crypt width ($P < 0.01$) respectively, was observed in the treated group when compared to control group. The oregano essential oil supplementation effectively improved intestine histology by promoting the villus and crypt growth.

Keywords: oregano essential oil, broiler, histology, small intestine

S1- 0290 Effect of oxidized wheat gluten on gut functions of broiler

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This experiment was conducted in order to investigate the effect of oxidized wheat gluten on the GUT structure and functions of broilers. 180 male Arbor Acre broilers were divided into three treatment groups including the control (C), the wheat gluten (G), and the oxidized wheat gluten (OG). We determined the pH value and moisture content of stool in each week, the growth performance, the apparent digestibility of protein in stool, ileum morphology at 21 day and 35 day. Data was analyzed by the one-way ANOVA procedure of SPSS. Animal fed 8% oxidized wheat gluten developed mild diarrhea that persisted throughout the whole experiment period, and OG treatment significantly reduced the pH value and increased the moisture content of stool in each week ($P<0.05$). The BW, ADG, ADFI were significantly lower in OG treatment compared with C treatment ($P<0.05$). Feed conversion ratio was higher in birds fed the OG diet compared with those fed the C diet ($P<0.05$). OG treatment significantly reduced the apparent digestibility of protein of 21- and 35-day-old broilers. However, the ileum morphology did not differ across all the treatments. Oxidized wheat gluten induced diarrhea of broiler without altering the ileum morphology.

Keywords: oxidized wheat gluten, diarrhea, growth performance, gut, broiler

S1-0291 Enhancement of egg quality and yolk color by carotenoids from corn distillers solubles oil and commercial tegetes extract pigments

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Egg yolk color is an important qualitative factor that affect for consumer preference depending on the country or the word region. Many cultures prefer an intense yellow pigmentation of the yolk, and it is also indicative of good enteric health of laying hens, since all of the pigments deposited in the yolk are of dietary origin. The bioavailability of dietary pigments may vary depending on the source of the feed ingredient, carotenoids from tegetes extract being the most commercially concentrated. However, corn distiller's solubles oil (CDSO) may serve as a cost-effective alternative of natural carotenoids to enhance yolk color and egg quality. The objective of this study was to determine the bioavailability of CDSO carotenoids for yolk pigmentation relative to other commercial pigments sources. A Basal diet, containing white corn and soybean meal, was supplemented with 0, 50, or 100% CDSO in place of 2% added soybean oil. To serve as positive controls, 2 dietary tegetes extract products (Pixafil LZ, Alcosa Biotec, S.A., Apaseo El Grande, Mexico; and Oro Glo 15, Kemin, Des Moines, IA) were added to the soy oil so as to supplement the same level of total pigments as contained in the 100% CDSO treatment. Forty 60-week-old Hy-Line W36 laying hens were randomly assigned among the 5 dietary treatments in individual cages and given ad libitum access to feed and water. After 2 weeks of adaptation to the experimental diets, feed intake, egg production, external and internal egg quality was determined for the following 7 weeks. During the last week, blood plasma was collected for total carotenoids content determination. There were no treatment effects on feed intake, egg production, or gEgg/gFeed. Yolk color index increased linearly as the level of CDSO increased, indicating similar bioavailability to the tegetes extract products. Carotenoid pigments from CDSO oil can be used to reduce tegetes extract supplementation for yolk pigmentation.

Keywords: egg, carotenoids, ddgs, tegetes, egg quality, laying hen, yolk color

S1-0292 Effects of probiotics on the performance and meat production of broilers fed low-fat distillers dried grains with solubles from 28 to 54 days of age

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The main and interactive effects of commercial probiotics (3 *Bacillus subtilis* species) and various levels of dietary low-fat distillers dried grains with solubles (LF-DDGS) on the Day 28 to 54 growth performance and meat production of broilers were evaluated. The apparent ileal digestible amino acid and apparent metabolic energy contents of the LF-DDGS were determined using 40 male Ross × Ross 708 broilers in a preliminary trial. Another 960 male Ross × Ross 708 broilers were fed a common diet containing 3 % LF-DDGS from Day 0 to 27 before they were randomly assigned to 8 replication floor pens within each of 12 treatment groups (10 chicks/pen) on Day 28. The 12 dietary treatments were assigned in a 3 (corn and soybean-meal basal diet, basal diet with antibiotics, and basal diet with probiotics) × 4 (dietary LF-DDGS levels: 0, 8, 16, or 24%) factorial arrangement. All diets were formulated to contain the same apparent metabolic energy and digestible amino acid levels. When compared to a 0% LF-DDGS diet, using 8% LF-DDGS in the broiler diets did not affect growth performance from Day 28 to 54 or meat yield on Day 54. When compared to a 0% LF-DDGS diet, including LF-DDGS at 16 % in the broiler diets lowered Day 28 to 42 body weight gain (BWG), Day 43 to 54 feed intake, Day 28 to 54 feed intake and BWG, and increased spleen weight on Day 54. In addition, increasing LF-DDGS in the diets up to 24% lowered the Day 28 to 54 BWG. Antibiotics increased gizzard pH in comparison to control and probiotic supplemented diets. However, growth performance was not affected by dietary antibiotic or probiotic inclusion. There was no interaction between LF-DDGS level and the type of feed additive on performance or carcass yield on Day 54. In conclusion, LF-DDGS can be included in broiler diets up to 8% without lowering growth performance or carcass yield.

Keywords: *Bacillus subtilis*, broiler, carcass, growth, distillers dried grains with solubles

S1-0293 Meal worm (*Tenebrio molitor*) as potential alternative source of protein supplementation in broiler

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The recent innovation in poultry industry is to identify and utilize alternative cheap animal origin protein sources in poultry feed. Present study determined the effect of meal worm supplementation on feed intake, body weight gain, feed conversion ratio (FCR), dressing percentage, and mortality, acceptability and HI antibody titer against Newcastle disease in broilers. Meal worm larvae were produced in the lab, killed with concentrated salt solution and dried in the incubator at 40 °C for 24 hrs. Proximate analysis, amino acid and mineral profile of the dried meal worm meal was conducted before use. A total of 120 day old broiler chicks were divided into 4 groups (WM-1, WM-2, WM-3 and WM-0) of three replicates each containing ten birds. The study used 4 treatments at inclusion levels of meal worm WM-1 (50g), WM-2 (100g), WM-3 (150g) and WM-0 (Control) and continued for four weeks. Proximate composition of worm meal showed crude protein value (45.83%), crude fats (34.2%) and ash content (3.51%), essential amino acid as lysine (4.51 ± 0.3) and Methionine (1.34 ± 0.4) with substantial amount of calcium 4.1gm/kg and phosphorus 7.06gm/kg. No significant effect was found ($p \geq 0.05$) on the mean feed intake. Body weight gain was significantly higher in all supplemented groups. Overall FCR was significantly ($P > 0.05$) higher for control group. Compared with other groups the decreasing trend of FCR was declining as (2.01 ± 0.01 to 1.75 ± 0.01) with the increasing level of meal worm meal. Dressing percentage was significantly ($P < 0.05$) higher for supplemented groups as compared to control. Non-significant differences were observed in acceptability, hemagglutination antibody titer against Newcastle disease and mortality among groups. It was concluded that meal worm meal could be safely used in broiler ration for better performance without any loss to antibodies titer and acceptability of chicken meat.

Keywords: worm meal, proximate analysis, amino acid analysis, broilers, overall performance

S1-0294 Improving the nutritive value of cottonseed meal with microbial enzymes for broiler chickens at different phases of growth

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A study was carried out to assess the effect of cottonseed meal (CSM) utilization on broiler performance at different growth periods (starter, grower, and finisher) supplemented with microbial enzymes. A total of 360 day-old Ross 308 male broiler chickens were randomly assigned to six dietary treatments, each replicated six times with 10 birds per replicate. Chickens were fed diets based on wheat/sorghum, soy bean meal (SBM)/canola meal (CM) ad-libitum. Cotton seed meal introduced at 4, 8 and 12 % of completed diet, in starter, grower and finisher diets respectively, supplemented with or without one of two new-age microbial enzymes AXTRA XB (composite xylanase and beta-glucanase) and Avizyme1502 (composite xylanase, protease, and alpha-amylase). At d10 and d24 broiler chickens raised on CSM supplemented with both microbial enzymes had higher body weight gain (BWG) ($P<0.05$) than those on other treatments, with the heaviest ($P<0.05$) birds observed in CSM with Axtra XB group. Feed conversion ratio (FCR) followed a similar trend as BWG during the starter phase, with lower and better ($P<0.05$) values recorded for CSM supplemented with Axtra XB group. In the same way, during the grower and finisher phases, FCR was significantly improved with lower ($P<0.05$) values recorded in the same CSM and Axtra XB group. Feed intake and relative visceral organs weights were similar ($P<0.05$) between the treatments in all growth phases. Absolute drumstick weight was highest ($P<0.05$) in the CSM and Avizyme group, while the lowest weight was recorded in the control and Axtra XB group. It can be concluded that CSM-containing diets when supplemented with Axtra XB improved BWG as well as FCR throughout the experimental period. Therefore, adding microbial enzymes to diets containing CSM can improve the performance of birds in all three growth phases. Furthermore, Axtra XB when added to the diets showed superior performance in all growth phases than Avizyme.

Keywords: broilers, cottonseed meal, microbial enzymes, axtra XB, avizyme

S1- 0295 Replacement value of cottonseed meal (CSM) for soybean meal in broiler chicken diets containing microbial enzymes

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This study investigated the effect of replacing soybean meal (SBM) with cottonseed meal (CSM) in wheat/sorghum-, soybean/canola-based diets supplemented with microbial enzymes for broiler chickens. Cottonseed meal was included at three levels - 0, 6 and 12 % of complete diets (replacing 0, 20 and 40 % of SBM) and augmented with three levels of Axtra XB - 0, 250 and 500 g/tonne of complete diets. Birds were allocated randomly to nine treatments, each replicated six times, with 10 birds per replicate. The feed intake (FI) results from d1-10 and d1-35 showed significant ($P<0.05$) linear decrease with increasing CSM level in diet. On the other hand, increasing Axtra XB enzyme level resulted in a significant decrease in FI at d25 ($P<0.05$) and d35 ($P<0.05$). There was interaction between CSM and enzymes on body weight gain (BWG) at d10 and d25 ($P<0.01$). Feeding the diet containing 6 % CSM and 250 g enzyme/tonne increased BWG by 5.4 % at d10, while the same amount of enzyme at 12 % inclusion of CSM in diet yielded a 4.2 % in BWG at d25. It was observed that including CSM in the diet increased BWG ($P<0.05$), with the highest gain recorded at the 6% level of CSM. Clearer significant effects ($P<0.05$) of enzyme inclusion on BWG were noticeable at d35. Broiler chickens offered CSM at 12 % inclusion rate with both levels of enzyme addition had better ($P<0.05$) feed conversion ratio (FCR) than other dietary treatments at d10, d25 and d35. Cottonseed meal at 12 % with Axtra XB at 250 g/tonne tended to have better FCR ($P<0.05$) at all phases of feeding. Enzyme inclusion significantly improved ($P<0.05$) FCR throughout the experimental period. A significant interaction ($P<0.05$) between CSM and enzyme was observed at d10 on FCR. Results obtained from this study suggest that CSM can replace up to 40 % of SBM in broiler diets when supplemented with the test enzyme, with improvement in gross performance.

Keywords: broiler chickens, cottonseed meal, Axtra XB

S1- 0296 Effect of feeding Mexican sunflower (*Thitonia diversifolia*) leaf silage meal on broiler production performance

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The objective of this research was to investigate the effects of Mexican sunflower (*Thitonia diversifolia*) leaf silage meal (MSLMS) in feed on broiler production performance. The materials used were Mexican sunflower (*Thitonia diversifolia*) leaf silage meal and eighty (80) one-day old Arbor Acres broilers chicks. The method used in this research was experiment in a completely randomized design (CRD). The treatment consisted of control diet (T0), and three levels of addition of MSLMS (T1 = 5% MSLMS; T2 = 10% MSLMS; and T3 = 15% MSLMS). Every treatment was repeated 4 times, if there was significant influence, it is followed by Duncan's Multiple Range Test. The results of this research showed that MSLMS significant influence ($P < 0.05$) feed consumption, body weight gain, feed conversion ratio and carcass weight. The use of MSLMS in the ration above 10% decrease production performance. It is concluded that the addition MSLMS on broiler ration can only be used up to 10%.

Keywords: Mexican sunflower (*Thitonia diversifolia*), silage, broiler, production performance

S1-0297 Effects of heat stress on the CYP7A1 signal pathway in the liver of broiler

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The purpose of this experiment was to investigate the effects of heat stress on the CYP7A1 signal pathway in the liver and fat metabolism of broilers. 48 twenty-eight days old male Arbor Acres broilers with similar weight and healthy were chosen in this experiment and the 48 broilers will be randomly allocated to four groups, with 12 chickens per group. The four groups were treated with heat stress for 72 hours in treatment group ($31 \pm 1^\circ\text{C}$), 72 hours in control group ($25 \pm 1^\circ\text{C}$), heat stress for 24 hours in treatment group ($31 \pm 1^\circ\text{C}$) and 24 hours in control group ($25 \pm 1^\circ\text{C}$). The results show that the treatment of 72 hours heat stress can significantly increase the relative expression of FATP-1 gene ($P < 0.05$), SREBP-1C gene ($P < 0.05$), APOB gene ($P < 0.05$), AMPK α 1 gene ($P < 0.05$) and LXR gene ($P < 0.05$) in the liver. At the same time, the treatment of 72 hours heat stress can not significantly affect the expression of FXR gene ($P > 0.05$) and CYP7A1 gene ($P > 0.05$) in the liver. Also the treatment of 24 hours heat stress can not significantly affect the expression of FATP-1 gene ($P > 0.05$), SREBP-1C gene ($P > 0.05$), APOB gene ($P > 0.05$), LXR gene ($P > 0.05$), FXR gene ($P > 0.05$), AMPK α 1 gene ($P < 0.05$) and CYP7A1 gene ($P > 0.05$) in the liver. Therefore, the gene expression of fatty synthesis regulatory factors and energy sensor and transducer of cellular metabolism was increased by 72 hours of heat stress in broiler liver. The treatment of 72 hours heat stress can activate the CYP7A1 signal pathway and energy metabolism in broiler liver, and it can also increase the expression of genes related to the synthesis of bile acid in the liver.

Keywords: broiler, heat stress, fat metabolism, CYP7A1, AMPK α 1

S1- 0298 Effects of *Candida krusei* LSA on growth performance, immune function, blood biochemical indices and manure deodorization of broilers

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Candida Krusei is a kind of yeast with ammonia nitrogen degradation ability that our research team screened out from chicken manure. This study was to evaluate the effects of fodder adding different level of *Candida Krusei* LSA on growth performance, Immune Function, blood biochemical indices and manure deodorization effect of broilers. A total of 240 1-day-old healthy Arbor Acres male broilers were randomly allotted to 4 groups with 6 replicates per group and 10 birds per replicate. The four diets included a basal diet without antibiotic and 3 experimental diets supplemented with *Candida Krusei* LSA of 107 CFU/kg, 108 CFU/kg and 109 CFU/kg, respectively, based on the basal diet. Broilers were slaughtered at 1~42 days old. Feed and water were access ad libitum. The results showed as follows: 1) the difference of ADFI, ADG, BW and F/G of each experiment group was not significant ($P > 0.05$). 2) *Candida Krusei* LSA supplemented in diets was no significant influence on thymus index and bursal index of each experiment group ($P > 0.05$), but LSA of 108 CFU/kg significantly increased spleen index ($P < 0.05$). 3) *Candida Krusei* LSA supplemented in diets significantly reduced the uric acid and ammonia level in blood ($P < 0.05$), but it has no significant influence on biochemical indices such as serum total protein, albumin and globulin ($P > 0.05$). 4) *Candida Krusei* LSA supplemented in diets was no significant influence on water content and total nitrogen of manure ($P > 0.05$), but LSA of 108 CFU/kg decreased the ammonia nitrogen content of manure significantly ($P < 0.05$). In conclusion, *Candida Krusei* LSA supplemented in diets has no adverse effect on growth performance, immune organs and blood biochemical indices; *Candida Krusei* LSA supplemented in diets can enhance the immune function of broilers, improve their nitrogen metabolism, decrease the ammonia nitrogen content in the manure, and reduce environmental pollution.

Keywords: candida krusei, broiler, growth performance, immune, blood biochemical indices, manure deodorization

S1-0299 In ovo nutrient supplementation improves the intestinal morphology and gut-associated genes expression in fasted broiler chickens

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The present study evaluates the effects of in ovo nutrient supplementation in early post-hatch (PH) feed deprived (FD) broiler chickens. On the 18th d of incubation, 200 eggs each were injected with either 0.50 ml of in ovo nutrients (IOS) or with 0.50 ml of saline water (Sham) or kept as un-injected control. The chicks hatched from the respective group were further divided in to immediate feed, 24 and 36h FD and reared in battery brooders with six replicates of eight birds each. At 24 and 36h PH, four birds from each treatment were killed and intestinal tissues (Duodenum, jejunum & ileum) were collected for morphological study. The total RNA was isolated from jejunum tissue and the cDNA was synthesized using standard protocol. The expression of gut associated genes like caudal type homeobox (Cdx), fatty acid binding protein (FABP) and excitatory amino acid transporter (EAAT) were quantified by real-time PCR using iQ5 cyclor. Data were analyzed in two way ANOVA taking treatments (IOS, Sham & un-injected) and period of fasting (0, 24 and 36h) as factors. The fertile eggs were of similar mean weight, but 20th d embryo weight and chick to egg weight ratio was significantly higher in the IOS group compared to sham and un-injected control. The villi height (VH) and width (VW) of duodenum, jejunum and ileum at 24 and 36h PH was significantly higher ($P < 0.05$) in IOS chicks than the sham and un-injected control, but decreased significantly with the increase in fasting period. The interaction between treatments and period of fasting was significant for VH and VW in duodenum and jejunum, where IOS chicks fasted for 24 or 36h had higher VH and VW than the control- immediate fed chicks. The expression of Cdx and FABP gene increased in IOS and 24h fasted chicks and that of Cdx and EAAT gene in IOS and 36h fasted chicks as compared to control-fasted chicks. It is concluded that in ovo nutrient supplemented chicks can withstand the early post-hatch feed deprivation effectively.

Keywords: In ovo supplementation, fasting, intestinal morphology, gut-associated gene expression, broiler chickens

S1-0300 Evaluation of calcium and phosphorus absorbability of calcium and phosphate sources in broilers

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A broiler study was performed to determine the ileal calcium (Ca) and phosphorus (P) absorbability of five Ca and P sources, three from animal (Delfos, Calfos, porcine bone meal) and two of mineral (MCP, DCP) origin. Delfos is a dicalcium phosphate, and Calfos a tricalcium phosphate (hydroxyapatite). The WPSA protocol for determination of precaecal P digestibility was used. During the first 14 days, birds were housed in floor pens bedded with wood shavings and received ad libitum a standard broiler diet. At d14, broilers were randomly assigned to 38 floor pens (0.9 m², 10 birds/pen) with a slatted floor. From d14 onwards, one of the six experimental diets (a basal diet, and 5 diets containing the Ca and P sources) was provided. Test diets were replicated six times, and the basal diet eight times. Spatial structure of the test products was determined by electron microscopy. Diets met or exceeded CVB (2012) requirements for all nutrients except for Ca and P. The calculated total P (tP) content, phytate P and Ca content of the basal diet were 1.6 g/kg, 0.6 g/kg and 2.3 g/kg, respectively. The calculated P content of the test diets was 3.6 g/kg and the Ca:tP ratio was 1.4:1, except for the diets with Calfos and bone meal, which had calculated Ca: tP ratios of 1.58 and 1.49, respectively. At d24, all birds were euthanized, where after the content of the terminal part of the ileum was sampled. Ca and P digestibility were calculated by linear regression. Ca digestibility of MCP, DCP, Delfos, Calfos and bone meal amounted 80.4, 68.9, 86.4, 66.5 and 62.4%, respectively. P digestibility of the tested products was 88.5, 82.4, 94.5, 86.9 and 78.2%, respectively. Based on images of the electron microscopy, positive relationships between surface area and degree of crystallinity of test products and the determined P digestibility were observed.

Keywords: calcium, phosphorus, broiler, digestibility

S1-0301 The effect of dexamethasone and dietary energy on gene expression of appetite and energy in hypothalamus

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An experiment was conducted to investigate the effect of dietary energy level on the gene expression of hypothalamic appetite regulatory peptides. A total of 216 one-day-old male broiler chickens were divided into three groups. One group received a high energy (HE) diet, one group received a low energy (LE) diet and another received NRC diet for 31 days. At 28 days of age, the chickens from each group were further divided into two subgroups and received one of the following two treatments for 3 days: (1) subcutaneous injection of dexamethasone (DEX), once per day (DEX group; 2 mg of DEX/kg BW in saline) and (2) subcutaneous injection of saline, once per day (Control/Sham treatment group). At 31 days of age, samples of hypothalamus were obtained. In the results, for the broilers fed with low energy, DEX had no influence on the expression of hypothalamic LKB1, NPY, AMPK α 2, CCK, GR gene ($P > 0.05$); The expression of hypothalamic LKB1, NPY, CCK was significantly upregulated by DEX injection for NRC diet group ($P < 0.05$). High energy on the diet of broiler chickens, DEX injection increased the mRNA level of hypothalamic LKB1, NPY ($P < 0.05$). The present study showed that DEX injection had no effect on hypothalamic appetite regulatory peptides in low energy diet, but in high energy diet and NRC diet, it increased the gene expression of appetite regulatory peptides.

Keywords: broiler, hypothalamus, diet energy, dexamethasone, appetite

S1- 0302 Effects of multi- carbohydrase and phytase on digestibility of nutrients, energy and amino acids of corn in broiler diets

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Apparent digestibility of nutrients (ADN), metabolizable energy (ME) and apparent (AID) and standardized ileal (SID) amino acid digestibility in broiler chicks fed corn with or without multi-carbohydrase (MC; 35 U/g α -galactosidase, 110 U/g galactomannanase, 1500 U/g xylanase and 1100 U/g β -glucanase) and Phytase (Phy; 10000 FTU/g) were investigated. A total of 245 male broilers (7 birds/pen) were fed one reference diet (RD) and four corn-based and corn-starch-based diets containing 30% of corn in 2 (MC; 0 or 200 mg/kg) x 2 (Phy; 0 or 50 mg/kg) factorial arrangement. RD (5% casein) was used to estimate endogenous AA losses. Excreta samples were obtained from d 16 to 18, and all birds were killed on d 21 for ileal digesta, pancreas and liver collection. Dietary enzymes addition increased ($P < 0.05$) ADN, ME and AA, without effect ($P > 0.05$) on liver and pancreas weight. Interaction ($P < 0.05$) from MC x Phy was observed on CP, AID for Ile, Lys, Thr, Gly, Ser and Tyr, and SID for Ile, Leu, Lys, Cys, Glu, Ser and Tyr, and a trend for DM, ME and SID for Met, Phe and Ala. Phy improved ($P < 0.05$) the digestibility of minerals, Ca, P, AID for Arg, His, Leu, Met, Val, Asp and Cys, and SID for Arg, His, Thr, Val and Asp. MC had positive effects ($P < 0.05$) on NDF, AID for Arg, His, Met, Phe, Val and Asp, and SID for Arg, His, Val, Asp, Gly and Pro. The SID means for 17 AA were: non-enzymatic diets, 89.46%; MC, 90.51%; Phy, 91.05%; and MC + Phy, 91.38%. The beneficial effects of MC and Phy with corn combination may be an effective nutritional strategy for improve nutrients, ME and AA use. Corn composition may be reviewed for enzymes uses in broiler.

Keywords: nutrients, enzymes, energy

S1- 0303 Digestibility of sunflower meal with enzymes supplementation in broiler diets

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Apparent digestibility of nutrients (ADN), metabolizable energy (ME) and apparent (AID) and standardized (SID) ileal digestibility of amino acids in broiler chicks fed sunflower meal (SFM) with or without multi-carbohydrase (MC; 35 U/g α -galactosidase, 110 U/g galactomannanase, 1500 U/g xylanase and 1100 U/g β -glucanase) and Phytase (Phy; 10000 FTU/g) were investigated. A total of 245 male broilers (7 birds/pen) were fed one reference diet (RD) and four corn-based and corn-starch-based diets containing 30% of SFM as sole source of protein in 2 (MC; 0 or 200 mg/kg) x 2 (Phy; 0 or 50 mg/kg) factorial arrangement. RD (5% casein) was used to estimate endogenous AA losses. Oxide of chromium (0.3%) was used as indigestible marker. Excreta samples were obtained from d 16 to 18. All birds were killed on d 21 for ileal digesta, pancreas and liver collection. Dietary enzymes supplementation coincided ($P < 0.05$) with ADN increase, ME and AA and liver and pancreas weight. Interaction ($P < 0.05$) from MC x Phy was observed on DM, ME, CP, AID of Arg, His, Lys, Met, Cys, Glu, Ser and Tyr, and SID of His, Ile, Leu, Lys, Met, Thr, Asp, Cys, Glu, Gly, Ser and Tyr. Phy improved ($P < 0.05$) the digestibility of minerals, Ca, P, AID of Ile, Phe, Ala, Gly and Pro, and SID of Phe, Val, Ala and Pro. MC had significant ($P < 0.05$) effects on NDF, AID of Ile, Phe, Val, Ala and Gly, and SID of Phe, Val, Ala and Pro, and liver and pancreas weight. The SID means for 17 AA were: non-enzymatic diets, 84.36%; MC, 85.23%; Phy, 85.91%; and MC + Phy, 86.18%. The combination of MC and Phy may be an effective nutritional strategy for improve nutrients, ME, CP, minerals and AA use in SFM for broiler diets.

Keywords: multi-carbohydrase, phytase, amino acids

S1-0304 Effects of sea buckthorn flavones on intramuscular fat deposition and lipometabolism for broilers

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This study was to evaluate effects of sea buckthorn flavones (FSB) on intramuscular fat (IMF) deposition and lipometabolism for broilers. A total of 240 1-day-old Arbor Acres male broilers were randomly allotted into 2 groups with 6 replicates of 20 broilers in each by a single factor design of experiment. The diets concluded a basal diet and a trial diet added 0.10% FSB. Broilers had access to feed and water ad libitum throughout whole study period of 42 days. The data were analyzed by SPSS 19.0 software package for Windows. At d 7, 14, 21, 28, 35 and 42 of trial, blood, breast and thigh muscles of broilers were sampled for analysis. The results indicated that during prior period of growth, IMF contents in breast and thigh muscles appeared V trend, higher at d 7, decreased at d 14, subsequently increased at d 21, however, upward trend for breast muscle and downward trend for thigh muscle during later period. Thigh muscle had higher IMF than breast muscle. Triglyceride and cholesterol contents in serum of broilers gradually decreased with age, but very low-density lipoproteins (VLDL) content gradually increased. FSB significantly improved IMF content in breast meat at d 28, 35, 42 and thigh meat at d 7, 14, respectively ($P < 0.05$). FSB obviously decreased triglyceride content at d 42 and low-density lipoprotein content at d 28, increased high-density lipoprotein content at d 28, 35 and VLDL content at d 14 and 42 in serum of broilers ($P < 0.05$). In conclusion, with increasing ages, IMF deposition in breast and thigh muscles had a waving trend. The VLDL and triglyceride contents in serum of broilers were closely related to IMF deposition. FSB changed triglyceride content in serum through modulating VLDL content, then regulated IMF deposition. The regulatory effect on IMF in breast muscle was more significant than that in thigh muscle, which indicated breast muscle was more sensitive to FSB.

Keywords: sea buckthorn flavone, broiler, intramuscular fat, lipometabolism

S1-0305 Effects of dietary lysine level on productive performance and nitrogen metabolism of Linwu ducks in peak laying period

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This experiment was conducted to study the effects of dietary lysine (Lys) levels on productive performances, egg quality, serum biochemical indices and nitrogen metabolism of Linwu ducks aged from 30 to 38 weeks, and to evaluate Lys requirement of Linwu ducks during the peak laying period. A total of 200 healthy Linwu ducks with similar body weight and laying rate were randomly assigned to 5 groups with 5 replicates of 8 ducks in each, and fed diets with 0.65%, 0.75%, 0.85%, 0.95%, 1.05% Lys respectively. Ten ducks with an average weight of each group were picked up for metabolism experiment at the end of the feeding experiment. The results showed as followed: 1) Daily egg yield was higher ($P < 0.05$) and feed/egg was lower ($P < 0.05$) at dietary Lys level of 0.95% and 1.05% than those in 0.65% and 0.75% Lys group. 2) Egg quality was not significantly affected by treatments ($P > 0.05$). 3) Total protein content in serum was significantly improved in 0.85%, 0.95%, 1.05% Lys groups compared to 0.65% Lys group ($P < 0.05$). Serum T3 content increased significantly at 0.95% Lys group compared with that in 0.65%, 0.75% and 1.05% Lys groups ($P < 0.05$). 4) The NPU and nitrogen deposition were maximized after feeding supplemental 0.95% Lys. According to the quadratic regression analysis based on total protein, NPU and nitrogen deposition, the optimal dietary levels of Lys for Linwu ducks were 0.92%, 0.96% and 0.95%, respectively. Daily egg yield, feed/egg, total protein, NPU and nitrogen deposition of Linwu ducks aged from 30 to 38 weeks were more sensitive to changes of dietary Lys level. The optimal dietary Lys levels for Linwu ducks is 0.92% to 0.96%, that can obtain the best performance, serum biochemical indices and nitrogen metabolism.

Keywords: lysine, laying duck, productive performances, nitrogen metabolism

S1- 0306 Synergistic efficacy of formic acid and *Origanum vulgare* in broiler diets

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An experiment was conducted to evaluate the synergistic efficacy of formic acid and *Origanum vulgare* in broiler diets. Experiments were carried out as a completely randomized design in a factorial arrangement (3×3) with 4 replicates of 10 chicks in each pen. Factors included 3 concentrations of formic acid (0, 1% and 2%) and 3 concentrations of *Origanum vulgare* powder (0, 1% and 2%). BW and cumulative feed intake were measure weekly. Blood characteristics, relative weight and length of different parts of the carcass, gastrointestinal pH, villi length, and crypt depth were measured at 42 d of age. Addition of 2% formic acid significantly increased BW gain at first week and also during the 14 and 21 d of age but had no obvious effect on the BW gain after 21 d of age. Formic acid supplementation did decrease FCR without any consequence in feed intake during the experimental period ($P \leq 0.05$). The inclusion of *Origanum vulgare* had no significant effect on BW, feed intake and FCR during the experimental period. The inclusion of *Origanum vulgare* linearly decreased pH of gizzard, duodenum, jejunum, ileum but increased pH of caeca content ($P \leq 0.01$). Addition of 2% formic acid significantly decreased pH of gizzard, duodenum, jejunum, ileum but increased pH of caeca content ($P \leq 0.01$). The synergistic effect of formic acid and *Origanum vulgare* on pH of different part of the gastrointestinal content of broiler was obvious. There was no significant interaction of formic acid and *Origanum vulgare* on performance characteristics of broiler fed both of this products. Results of current study showed that using mix of the organic acid and phyto-genic product could make a healthy intestine in broiler chicks.

Keywords: formic acid, *Origanum vulgare*, performance

S1- 0307 Evaluate requirements of Cu, Fe, Zn and Mn for broilers using uniform design method

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Two experiments were conducted to research the ideal balance profile of Cu, Fe, Zn and Mn for 1-21 or 22-42 day-aged boilers via Uniform experimental design method. In Experiment 1, 1-day aged male broilers ($n=900$) were allotted to 15 groups with 6 replicates of 10 birds. A basal diet containing 8 mg/kg Cu, 123 mg/kg Fe, 40 mg/kg Zn, 60 mg/kg Mn and 14 experimental diets formulated by adding 2~8 mg/kg Cu, 10~35 mg/kg Fe, 10~40 mg/kg Zn, 15~60 mg/kg Mn based on the basal diet were used to feed the corresponding groups for 1-21 d. In experiment 2, nine hundred male broilers aged 22d were allotted to 15 groups with 6 replicates of 10 birds. A basal diet (8 mg/kg Cu, 125 mg/kg Fe, 40 mg/kg Zn, 60 mg/kg Mn) and 14 experimental diets which were formulated by adding 2~8 mg/kg Cu, 10~35 mg/kg Fe, 10~40 mg/kg Zn, 15~60 mg/kg Mn based on the basal diet were fed to corresponding groups to 42 d. The growth performance of broilers and mineral excretion from their manure were used to reckon the ideal balance profile of Cu, Fe, Zn and Mn for different feeding period. The results showed that dietary treatments affected ADG of 1-21 d broilers and mineral excretion of 1-21 d or 22-42 d broiler significantly. Obvious interaction was observed between Cu, Fe, Zn, Mn for mineral excretion. Regression analysis found that for 1-21 d broilers, when the levels of Cu, Fe, Zn and Mn were 16.96, 166.66, 46.01 and 60.26 mg/kg, ADG got optimum value. The total excretion of Cu, Fe, Zn and Mn got the minimum when dietary level of Cu, Fe, Zn, Mn was 8.54, 130.66, 38.19, 64.07 mg/kg. For 22~42 d broiler, when the total mineral excretion got the minimum value, the optimum combination of Cu, Fe, Zn, Mn were 7.36, 125.74, 38.23, 62.76 mg/kg. It was concluded that there is a dilemma between growth and mineral excretion. To maintain appropriate proportion of dietary level of Cu, Fe, Zn and Mn could reduce mineral excretion without negative effect on growth of broilers.

Keywords: trace mineral, broiler, growth performance, excretion, uniform design, balance profile, interaction

S1-0308 Evaluation of dietary calcium requirement of Linwu laying ducks aged from 22 to 28 weeks

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This study investigated the effects of dietary calcium levels on laying performance, egg quality, tibia quality and serum biochemical indices of Linwu ducks aged from 22 to 28 weeks, and then to estimate the calcium requirement for diets of Linwu ducks. A total of 200 laying ducks were randomly divided into 5 treatments with 5 replicates of 8 ducks in each, and fed diets with 1.50%, 2.00%, 2.50%, 3.00%, 3.50% calcium, respectively. The results showed as follows: 1) Calcium level had no effect on feed consumption or laying rate. Increasing dietary level of calcium from 1.50% to 3.00% in duck's diets in increment of 0.50% had a significant positive linear effect on egg mass, daily egg yield, qualified rate of egg and feed/egg ($P < 0.05$). 2) Calcium level had no significant effects on egg quality of laying ducks ($P > 0.05$). 3) The dietary 1.50% calcium level group displayed the lowest brooding strength, calcium content in ash and calcium content in defatted tibia, while the best results of tibia indices were recorded from the fourth group feed diet containing 3.00% calcium. 4) Different dietary calcium levels had no significantly effect on the contents of calcium and phosphorus in serum of ducks ($P > 0.05$). The serum alkaline phosphatase (AKP) activity in 1.50% calcium group significantly higher than the other groups ($P < 0.05$). 5) According to the quadratic regression analysis based on average egg weight, calcium content in ash and calcium content in defatted tibia, the optimal dietary levels of calcium for Linwu ducks were 2.79%, 2.98% and 2.98%, respectively. High level of dietary calcium (3.00%) has improved egg production, serum biochemical indices and tibia quality. A percentage 2.79%~2.98% of calcium in the diet is recommended for Linwu laying ducks in earlier period of production.

Keywords: calcium, egg production, egg quality, tibia quality, Linwu ducks

S1-0309 Organic acid application in drinking water for broiler breeder improves farm biosecurity and intestinal health—a field trial in west java, Indonesia

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Pathogen bacteria contaminated drinking water was treated with an organic acid blend of free and buffered short-chain fatty acids (formic and acetic acid, ammonium formate) and copper to reduce bacteria loads in a commercial closed house broiler breeder farm in West Java, Indonesia, for 34 days. Before treatment, the 10,000 Lohman broiler were affected by yolk peritonitis, diarrhea, necrotic enteritis and respiratory deficiencies. Before and after treatment, samples from the drinking water lines were taken for pH and water hardness determination. Likewise, birds were randomly sampled for necropsy of gut scoring lesions. Mortality and dirty egg occurrence were monitored during the trial. Taking an organic acid blend treated drinking water of pH4 strongly reduced total bacteria counts from 80 CFU ml⁻¹ of water to 40 CFU ml⁻¹ in the lines and nipple drinkers to the birds. Coliforms reduced from 3 to 0 at the nipple drinkers. Clostridia were steadily determined lower than 101 CFU ml⁻¹ while no salmonella were recorded during the trial. Before treatment decaying level of intestinal tone, cellular sloughing and mucus level were very high based on ocular observation, both parameters reduced after organic acid drinking water treatment. Likewise guts were less thin. Dirty eggs counts reduced during organic acid drinking water treatment with concomitant lesser contamination level of hatching eggs and increased egg quality production leading to a higher number of chicks. Although mortality did not differ significantly during the treatment period of drinking water with organic acids, medication costs were reduced. Treating poultry farm drinking water with the organic acid blend product Selko pH® is recommended during 3 days weekly for 24 hours to continuously suppress pathogen bacterial loads in the water lines and intestinal tract as well as keep birds drinking continuously to maintain a strong growth and health performance.

Keywords: organic acids, drinking water, broiler

S1-0310 Effects of Bile acids on performance of broiler

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The aim of the study was to research the effects of bile acid on broiler production performance, blood indicators and slaughter performance. In the experiment, a total of 2400 one-day-old AA broilers were randomly allotted into four treatments with six replicates per treatment. Treatment 1 was female broilers fed with basal diet, treatment 2 is male broilers fed with basal diet, treatment 3 is female broilers fed with basal diet added bile acids, treatment 4 is male broilers fed with based diet added bile acids. The effects of bile acid on production of 21-day-old and 35-day-old broilers were investigated in the experiment. At 36 days, two trial broilers were slaughtered in every replicate of one treatment to investigate effects of bile acid on blood indicators and slaughter performance of broilers. The results showed: Compared with control, Adding bile acid to basal diet improved the EPI of female broilers significantly ($P < 0.05$), the male broilers had no significant effect on EPI ($P > 0.05$); TCHO level of female broilers significantly enhance ($P < 0.05$), impact on the male is not significant ($P > 0.05$); for female broilers, abdominal fat, eviscerated and eviscerated ratio has improved significantly ($P < 0.05$), but the effect is not obvious on male ($P > 0.05$).

Keywords: bile acid, production performance, blood indicator, slaughter performance

S1-0311 Effect of lactic acid bacteria on the growth performance and immune function of broilers fed diets contaminated with aflatoxin B1

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Aflatoxin contamination is very common in feed-stuffs across the world and finding an ideal detoxifier is urgent given the toxic action on animals and negative cascades on the food, human and environment, as a result of which the mechanism of detoxification is experiencing from the physical to biological method in order to thoroughly destroy the toxin. This study aims to investigate the effect of lactic acid bacteria (LAB) and hydrated sodium calcium aluminosilicate (HSCAS) on the growth performance, nutrient digestibility and immune function of broilers fed diets contaminated with aflatoxin B1 (AFB1). A total of 480 female Cobb broilers at one day old were randomly allotted into 4 groups with 6 replicates of 20 chicks each. Positive control (PC): no AFB1 in the diet; negative control (NC): containing AFB1 40 µg/kg; LAB: NC + LAB 1.5×10^{10} CFU/kg; HSCAS: NC + HSCAS 3g/kg. The trial lasted for 21 days. Results showed that the NC diet reduced ($P < 0.05$) feed intake and body weight gain, and feed efficiency, and diets supplemented with LAB or HSCAS improved ($P < 0.05$) the growth performance of birds, and the effect of LAB showed better than the case of HSCAS. There was an increase of 7.1% to 13.1% ($P < 0.05$) in the nutrient digestibility of energy, crude protein and crude fat in LAB group, and 3.2% to 8.5% ($P < 0.05$) in HSCAS group, and the digestibility of energy and crude protein increased ($P < 0.05$) by respective 4.1% and 4.5% in LAB compared to HSCAS. The indexes of thymus gland, bursa fabricius and spleen and contents of immunoglobulin A, M and G in LAB were 5.1 to 11.9% higher than that in HSCAS. The results suggest that LAB preparation in the present trial shows significant potential in the detoxification of AFB1 in broilers.

Keywords: aflatoxin B1, broiler, growth performance, immune function, lactic acid bacteria

S1-0312 Effects of turmeric residue on performance, egg qualities and yolk cholesterol content of layers

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The aim of this study was to determine the effects of turmeric residue (TR) on performance, egg qualities and cholesterol content in yolk of laying hens. Three hundred and eighty-four 56-week-old Roman brown laying hens were randomly assigned to four groups with six replicates per group and sixteen hens per replicate. The laying hens in group 1 (the control group) were fed a basal diet, and those in groups 2 to 4 were separately fed the basal diets with 0.5%, 1% and 1.5% TR. The experiment lasted for 63 days, including 7 days for pretest. The results showed as follows: compared with control group, adding 1.5% TR significantly increased the qualified rate of egg and decreased the soft-broken egg rate and second egg rate ($P<0.05$); adding TR could enhance the egg qualities, but have no significant difference ($P>0.05$); adding 0.5%~1.5% TR could reduce the contents of cholesterol and MDA in yolk ($P<0.05$). These results indicate that TR can increase performance and egg qualities of layers, and decline the contents of the cholesterol and MDA in yolk.

Keywords: Turmeric residue (TR); laying hen; performance; egg qualities; cholesterol; MDA

S1-0313 Effects of palm kernel meal and copra meal supplemented with compound enzymes on nutrient and energy utilization rates of Linwu ducks

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The aim of this study was to determine the effect of compound enzyme supplementation on nutrient and energy utilization in Linwu ducks fed Palm kernel meal (PKM) and Copra meal (CM). Forty healthy adult Linwu ducks with body weight of about 2.0 kg were randomly divided into 5 groups and 8 replicates in each group. Metabolic tests were carried out by hunger strike - gavage method. Ducks in groups 1 to 4 were fed 50 g PKM or CM diet without or with 250 mg/kg compound enzymes by gavage, respectively. Ducks in group 5 were fed 50 g diet with no nitrogen. The apparent and true metabolic energy (AME and TME), apparent and true utilization rate of dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and 17 kinds of amino acids of PKM and CM supplemented with compound enzymes in Linwu ducks were determined. The results showed as follows: supplemented with compound enzymes, the true utilization rate of CP in PKM and CM were significantly increased by 12.62% and 13.73%, respectively ($P<0.05$); TME increased by 5.61% and 3.63%, respectively ($P>0.05$); the true utilization rates of 17 kinds of amino acids increased by 0.27%~7.36% and 0.67%~4.99%, respectively; the true utilization rates of tyrosine and proline in PKM, aspartate, isoleucine, tyrosine and proline in CM were significantly increased ($P<0.05$). The study indicates that PKM and CM supplemented with compound enzymes containing protease and non-starch polysaccharide enzyme could improve utilization rates of nutrient and energy of Linwu ducks.

Keywords: Palm kernel meal, copra meal, nutrient utilization rate, metabolizable energy, compound enzymes

S1-0314 Effects of citric acid supplementation to laying hen diets in different levels of Ca and P on the performance and egg quality characteristics

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In this experiment, effects of citric acid supplementation (0 and 4 %) to laying hen diets with different Ca (3.0% and 3.5 %) and available P (Pa) (0.15% and 0.35 %) levels on the some egg quality characteristics. Twenty five weeks- old RIR x LINE 54 (ATAK) brown laying hen hybrids were used. Laying hens were randomly distributed to 32 pens with 40 hens of each. Experiment was carried out with 8 dietary treatments with 4 replicates in random block factorial (2x2x2) arrangement and continued from 25 to 60 weeks of age in two feeding periods. Laying hens were fed with isonitrogenous and isocaloric experimental diets of 17.50 % crude protein and 2770 kcal ME/kg for first period (25 to 41 weeks) and 16.75 % crude protein and 2750 kcal ME/kg for second period (44 to 60 weeks). Citric acid supplementation to diet of 3.5 % Ca and 0.35 % Pa increased laying rate ($P<0.05$). However, egg weight was higher for diet of 3.0 % Ca and 0.15 % Pa with citric acid ($P<0.05$). Egg mass was not affected by treatments ($P>0.05$). Albumen height and Haugh unit were reduced by citric acid supplementation at the 3.0 % Ca level ($P<0.05$). Effects of main factors on the egg yolk colour were not significant. As a result, citric acid supplementation to laying hen diets had significant effects on some performance traits without affecting the egg quality parameters.

Keywords: laying hens, egg quality, citric acid

S1-0315 Effect of split feed and delayed point of lay on production and egg quality

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Extension of the production cycle of laying hens becomes more and more important in the current farm management. The most important restriction factor hereby is the laying percentage and egg quality at later age of the hens. Therefore we investigated the effect of a split feed with different calcium contents in the morning and afternoon feed on the one hand and the effect of delaying the point of lay on the other hand. We performed the feed experiment in enriched cages and the light experiment in both enriched cages and aviaries. We aimed to extend the production cycle to at least 85-90 weeks however we were forced to stop at 70 weeks of age due to reasons not related to the hens or egg quality. Lohmann Brown Classic and Lohmann LSL Classic hens were kept in enriched cages and Lohmann Brown Classic hens were kept in aviaries. Until 70 weeks of age, the hens on the normal light schedule performed better compared to the hens where the point of lay was restrained. It is possible that a delayed light schedule on the laying farm is not sufficient and that the light schedule during the rearing period should also be adapted to a hen that reaches her point of lay later. For the feed experiment, we have seen a lower feed conversion and lower cumulative feed consumption in the hens that were fed the split feed. In addition, the brown hens on the split feed showed a lower percentage of cracked eggs. At the age of 70 weeks no differences were found between the groups that were fed the different feeds. Unfortunately we have no data after 70 weeks of age. We would expect to see a decreased laying percentage and egg quality at the age of 75-80 weeks of age. In conclusion we can say that delaying the point of lay and feeding a split feed does not have a beneficial effect for the farmer until 70 weeks of age. However, further research is needed on flocks that are kept until at least 90 weeks of age.

Keywords: extended production cycle, egg quality, light schedule, calcium

S1-0316 Gastrointestinal lipid classes changes according to dietary fat consumption in broiler chickens at different ages

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The aim of this study was to determine the influence of age and saturation degree of dietary fat on the lipid classes (LC) dynamics along the gastrointestinal tract (GT) in broiler chickens. 160 one-day-old broilers were randomly distributed in 2 treatments (8 replicates/treatment). A basal diet was supplemented with 6% of palm oil (P; saturated) or soybean oil (S; unsaturated). Two digestibility balances were carried out between 11-14d and 33-35d, and digestive content (gizzard, duodenum, jejunum and ileum) and excreta samples were collected to analyze LC by HPLC-RID. Fatty acids (FA) were analyzed by GC-FID. Data was analyzed by ANOVA. As lipids moved along the GT, the level of triacylglycerols (TAG) decreased, and the level of free fatty acids (FFA) increased, related with the hydrolysis of dietary TAG and diacylglycerols (DAG). At 14d, S resulted in a greater and faster production of FFA than P. The FFA highest level was reached at duodenum for S, and at ileum for P. This suggests hydrolysis process has a slower rate for a saturated fat. FFA decrease is related with its absorption and it was faster in S than in P. This was also reflected in excreta ($P < 0.05$). FFA content was correlated with the different apparent digestibility (AD) of total FA (S: 81.9 and P: 61.7%, $P < 0.001$). AD was higher at 35d than at 14d. This agrees with an improvement of the hydrolysis and absorption processes due to the age. This improvement was greater for P than for S, leading to a reduction of the differences between S and P observed at younger ages. Still, AD of total FA kept being higher for S than for P (S: 88.8 and P: 79.3%, $P < 0.001$). In conclusion, the different dietary fat utilization according to the age and fat unsaturation degree is related with the LC changes observed along the GT.

Keywords: fat digestion process, lipid classes, fatty acids, digestibility, broiler chickens

S1- 0317 Effect of split feeding on performance and egg quality in aged laying hens

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The effect of 1 conventional and 5 split feeding treatments was investigated on performance and egg quality of individually housed Dekalb White hens (75-90 wk, $n=12$ /treatment). The control treatment (T1) received the same feed in the morning (M) and in the afternoon (A), which contained fine (F) and coarse (C) limestone (LS) at ratio 50:50. For the split treatments, the ratio of FLS and CLS was 50:50 or 30:70 and time of administration (M/A) differed. Following treatments were given: T2=50CM:50FA, T3= 30FA:70CA, T4=50FM:50CA, T5=30FM:20FA+50CA and T6=30FM:70CA. Due to an unexpectedly low FI and laying%, T1 could not be compared to the split treatments and was excluded from the analysis. Laying% of hens in T4 (89.6%) and T5 (92.9%) was higher than in T2 (66.1%) at 89-90 wk of age ($P \leq 0.05$). Laying% of T3 (71.7%) was lower than that of T5, and T6 (86.8%) did not differ from the other treatments. Feed conversion ratio (FCR) in T2 was lower than in T5, T3 and T6 at 87-88 wk ($P \leq 0.05$). At 89-90 wk FCR of T3 increased, and T2 and T3 had a higher FCR compared to T4, T5 and T6. EW in T3 was higher than that in T2 at 78-82 and 86-88 wk of age ($P \leq 0.05$). For T6 this difference was significant at 80 and 86-88 wk. Eggs weighed 60.4g in T2 whereas EW was 66.3g in T3 and 67.3g in T6 at 88 wk. Shell thickness (ST) decreased from 403.2µm to 390.2µm between 76- 90 wk of age ($P=0.016$). Highest ST was obtained in T4 (408.0µm) and T5 (401.7µm) whereas ST of T2 (388.2µm) and T3 (394.3µm) was the lowest ($P=0.058$). These results indicate that time of feeding FLS and CLS have an effect on production and shell quality. When only FLS was fed in the afternoon (T2) shell formation had to be supported by bone reserves during the night which resulted in low ST. When all LS was fed in the afternoon (T3) EW increased and ST decreased. Whereas providing FLS in the morning and CLS in the afternoon in ratios of 50:50 (T4), 30:20+50 (T5) or 30:70 (T6) resulted in favourable performance and shell quality.

Keywords: aged laying hen, split feeding, egg quality

S1-0318 The Effect of a coated calcium butyrate complex on the oviduct of broiler breeders

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This trial was done to determine the effect of a coated calcium butyrate complex (CCB) on structure and function of the reproductive tract in broiler breeders, eggshell quality and hatchability of eggs. Six houses with 10.000 Ross 308 breeders each were divided in 2 treatment groups. Group 1 received a control diet and group 2 a CCB supplemented diet. The birds were fed specific diets from week 18 until 61 of age. After this period, 15 birds per group were culled and the reproduction tract was collected for gross anatomical and histological analysis. Thirty eggs per treatment underwent quality measurements. Hatchability data from all houses were registered. Anatomical measurements on the birds on CCB resulted in significantly higher reproductive tract weight/body weight (%). The uterus was numerically longer in group 2. Also, the number of mature ova was significantly higher in birds fed CCB with numerically heavier ovaries. On histological analysis revealed significantly wider primary folds in the uterus. The breaking strength of the eggs from birds in group 2 was numerically stronger and in the hatchery 5 more eggs per breeder was. With this trial we can conclude that the reproductive system was better developed in the breeders fed CCB and that the higher number of mature ova can be consistent with greater persistency in lay. Also, significant difference was observed for the breadth of the primary folds in CCB group. This suggest an increase in the available surface area of the primary folds of the uterus and consequently the area for ion transport during shell formation. The latter can explain improvement in breaking strength of the eggshell in this group.

Keywords: butyrate, oviduct, broiler breeders

S1-0319 Influence of dietary fats on the fatty acid composition of uropygial gland secretion in broiler chickens

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Studies have shown that the biochemical composition of uropygial secretion (or preen oil) of birds, particularly water-fowl, is affected by various factors including species, gender, season and age. However there are few such studies in chickens. To improve meat quality of broilers it is common to manipulate the fatty acid composition of the diet but to determine the effectiveness of the dietary manipulation requires invasive blood sampling or humane slaughter of the birds. In this study, we tested the hypothesis that the fatty acid composition of preen oil could provide a quick, simple and non-invasive way of assessing the fatty acid profile of chickens. Cobb 500 broilers of both genders were fed a diet supplemented with 4% (w/w) flaxseed oil (high n-3 polyunsaturates) or beef tallow (mostly monounsaturates and saturates) for 6 weeks. Preen oil (squeezed from preen gland) and whole blood samples (n=36) were collected post mortem for fatty acids analysis by Gas Chromatography. The results of preen oil analysis showed that about 97% of fatty acids were saturates with traces of monounsaturates and polyunsaturates (mostly omega-6). There were negligible n-3 polyunsaturates in preen oil even though these were significantly higher in the blood of birds on the flaxseed oil diet. Some saturated fatty acids were slightly, but significantly, affected by diet (C16:0, C17:0 and C22:0) or by gender (C10:0 and C18:0) ($P \leq 0.05$); however the effect of the diet by gender interaction on fatty acids composition was not significant ($P > 0.05$). Some fatty acids with odd numbers of carbon atoms (such as C19:0) were found in relatively high concentrations in preen oil, despite not being detectable in the diets or blood. This suggests that the microbiota in the uropygial gland was most likely responsible for their synthesis from other fatty acids. In conclusion, the fatty acid composition of preen oil does not accurately reflect the fatty acid profile of the diet or blood of broiler chickens.

Keywords: fatty acids, broilers, diet, preen oil, blood

S1-0320 Emulsifier improves energy utilization in broiler chickens

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The objective of this study was to evaluate the effects of an emulsifier-additive on nutrient digestibility and nitrogen-corrected apparent metabolizable energy (AMEn) in broilers receiving diets with different soybean oil levels in the starter (14-21d) and finisher (35-42d) phases. Two trials were conducted using mash corn/soybean-based diets with or without inclusion (350g/ton) of emulsifier (Excential Energy Plus) and five levels of soybean oil (0; 1.5; 3.0; 4.5; 6.0%). A total of 960 male Cobb 500 in the starter and 360 male broilers in the finisher phase were allocated (metabolic cages) in a complete randomized design with 10 treatments and 6 reps each. Total excreta were collected from d 19 to 21 and from d 40 to 42 to determine AMEn, apparent digestibility coefficients for dry matter (ADCDM) and crude protein (ADCCP). Data were analyzed using ANOVA (PROC GLM/SAS) and CONTRAST test among treatments. In the starter phase, there was no effect ($P>0.05$) for ADCCP, but there were effects ($P<0.05$) of using emulsifier on ADCDM; treatments with 3.0 and 6.0% of oil had higher values (74.96 vs 72.91; 73.99 vs 72.19). AMEn was improved ($P<0.05$) by 61, 65 and 70 kcal/kg when emulsifier was used in the treatments with 3.0, 4.5 and 6.0% of soybean oil. In the finisher phase, there was no effect ($P>0.05$) for ADCCP. However, emulsifier increased ($P<0.05$) ADCDM in the treatments with 4.5 and 6.0% of oil (76.31 vs 74.14; 74.34 vs 72.38). The AMEn was improved ($P<0.05$) by 81, 87 and 99 kcal/kg when emulsifier was used in the treatments with 3.0; 4.5 and 6.0% of soybean oil. In conclusion, Excential Energy Plus can significantly improve AMEn when higher levels of oil are used in the diets.

Keywords: feed additive, broiler, nutrient digestibility, energy, emulsifier

S1-0321 Effect of protected sodium butyrate and nutrients concentration on broilers performance

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The study was conducted to compare the effect of sodium butyrate protected with sodium salt of palm fatty acids distillates (GUSTOR N'RGY) with diets differing in nutrient concentration on growth performance. A 2 x 2 factorial design was used with two basal diets based on corn and soybean meal: S (standard nutrient diet) and L (low nutrient diet) with a reduction of 60 Kcal/Kg of ME and 2.3% lower concentration of aminoacids; with or without addition of GUSTOR N'RGY at 1 kg/t (B, C). A total of 160 Cobb one-day-old chickens were randomly allocated to 4 treatments with 4 floor pens of 10 birds per treatment: SC (standard diet), SB (standard diet + protected butyrate), LC (low diet), LB (low diet + protected butyrate). Mash feeds and water were offered ad libitum. Performance was recorded at 0, 21 and 42d. Data were analyzed with two-way ANOVA using the GLM procedure of SAS. FCR was affected by nutrient concentration by 2.5% ($P<0.05$); it showed also a tendency in the 0-21d phase, being the lowest values for diets with standard energy level (1.39 vs 1.35). However, it did not affect any other parameters. Additive inclusion improved final BW and ADG (2.059 vs 2.205 kg, $P<0.05$) and ADG (48 vs 51 g, $P<0.05$). Furthermore, those differences were also observed in feed intake, where animals fed B diets ate 5g/d more than those fed C diets ($P<0.05$). As a consequence, there were no effects on FCR. Despite there were non-significant interaction between additive and nutrient concentration, a tendency ($P<0.10$) has been observed in feed intake at 21d being the lowest value for diet SC. It can be concluded that the addition of protected sodium butyrate improves performance (7% higher BW) in broilers. Besides, a 2.3% dilution of dietary nutrient concentration penalizes FCR in a 2.5%.

Keywords: protected sodium butyrate, energy, aminoacids

S1-0323 Effect of organic zinc and manganese on layer breeder performance

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The objective of the present study is to evaluate the effects of organic zinc and manganese on the reproductive performance of layer breeders. 576 healthy Hy-line brown parent layer breeder with similar body weight (CV <8%) will be selected at 23 wk of age and allocated to 3 treatments, with 12 replicate per treatment and 16 layers per replicate raised in 8 contiguous cages (2 layers per cage). 30 healthy Hy-line brown parent rooster with similar body weight (CV<8%) will be selected at 23 wk of age and randomly allocated to 3 treatments with 10 replicates per treatment. Each rooster is deemed as a replicate. The control group fed with basis diet+Zn (80mg/kg) and Mn (80mg/kg) sulfate, the iso group fed with basis diet+ Zn (40mg/kg) and Mn (40mg/kg) sulfate+organic zinc (40mg/kg) and organic manganese (40mg/kg), the add on top group fed with basis diet+Zn (80mg/kg) and Mn (80mg/kg) sulfate+organic zinc (40mg/kg) and organic manganese (40mg/kg). In the study, roosters will be fed with the same diet as layers for each treatment. The experiment lasted for 32 weeks. The results were as follow: compared with the control group, laying rate in the on-top group was higher. The egg weight in iso group and on-top group were higher than that in the control group, but there was no significant difference among the groups. The feed to gain ratio in the control group was higher than that of every test groups. The qualified egg rate in the control group was lower than the iso group and the on-top group. In conclusion, dietary organic zinc and manganese had relatively better effects on layer breeder performance.

Keywords: organic zinc, organic manganese, layer breeder, egg, performance

S1-0324 Effect of methanolic extract of *Caesalpinia sappan* L. on antioxidant activity, growth performance and carcass characteristics in heat stressed broiler chicken

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An experiment was conducted to evaluate the effects of methnolic extract of *Caesalpinia sappan* supplementation on the antioxidant status and growth performance of broiler chicken during summer months. One hundred day- old broiler chicks were randomly assigned into 5 groups. Control group I was given basal diet and treatment groups II, III and IV were supplemented with methanolic extract of *Caesalpinia sappan* stem bark @ 100,300 and 500 ppm respectively and group V was supplemented with vitamin E @ 100 ppm as an antioxidant control through drinking water from day 0 to 8 weeks of age. All the *C. sappan* supplemented groups showed significantly lowered levels of malondialdehyde (MDA) and higher levels of reduced glutathione, GPx, SOD and Catalase in liver and kidney tissues as compared to control. The serum levels of ALT, AST, CK and cholesterol were significantly lower in treatment groups III and IV when compared with control. However, serum T3 levels showed no significant change among different groups. Groups IV and III showed higher weekly feed intake, body weight gain and feed conversion ratio compared to control group. Higher dressing yields and lower abdominal fat percentages were observed in group IV compared to control. It could be concluded that, supplementation of *C. sappan* extract improved antioxidant status and growth performance in broiler chicken and can be considered as a replacement for synthetic antioxidant in poultry diet in heat stress conditions.

Keywords: *Caesalpinia sappan*, melondialdehyde, antioxidant, growthperformance, heat stress

S1-0326 Effects of gut health enhancer and drinking water product on growth performance, litter quality, and gut microbiota of broilers

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A study was conducted to compare the effect of antibiotic growth promoters (AGP) with two Products: A and B on growth performance and health of the broilers. Product A is a gut health enhancer based on organic acids and plant extracts. Product B is a drinking water product based on stabilized organic acids mainly for water quality and chicken health. A total of 1,500 day-old chicks (Ross, male) were randomly allocated into 5 treatments with 15 replicates of 20 chicks each. The experiment was divided into 2 phases containing d1 to 21 (Phase 1) and d22 to 42 (Phase 2). There were 5 treatments including a basal diet (Control), basal diet+AGP (Colistin sulphate premix to provide 20 ppm Colistin sulphate), basal diet+Product A (0.15% in Phases 1, 0.1% in phases 2 respectively), basal diet+Product B (0.05% of drinking water in Phase 1, 0.15% in Phase 2), basal diet+Product A+B (0.15% + 0.05% in Phase 1, 0.1%+0.15% in Phase 2). Body weight and feed intake were recorded weekly throughout 42 d. The PCR-DGGE assessment was used to measure the bacterial diversity of cecal contents at 21d. The litter moisture and footpad lesion score was analyzed at d 21 and d 42. The results showed that Product A improved ($P < 0.05$) feed conversion ratio (FCR) compared with Control and AGP in Phase 1, but weight gain was not affected ($P > 0.05$). AGP or Product A had the better FCR than Control in period d1 to 28. At d21, adding Product B or Product A+B significantly reduced ($P < 0.05$) the footpad lesion score and the litter moisture compared with Control. Furthermore, AGP or Product A had the highest gut microbial diversity, evenness and richness of bands, with the lowest in the group with Product A+B. In conclusion, the present study indicated that birds fed Product A had a comparative advantage of the growth performance of the chickens in d1 to 28. Product A can be used as an alternative to AGP in broiler chicken diets, and Product B was able to improve the food pad health and litter quality.

Keywords: antibiotic replacer, broiler, growth performance, litter quality, gut microbiota

S1-0327 Analysis of epizootic situation regarding productive poultry's ectoparasites in Ukraine

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At this time poultry is one of the most progressive livestock industries in Ukraine. Productive poultry has common infectious and parasitic diseases. Important role, among the parasitic diseases, has diseases that cause by parasitic insects. Their persistence among the livestock leads to reducing of poultry's productivity and it can outbreaks infectious and parasitic diseases. Ectoparasites are vectors and reservoirs of these pathogens. The goal of our work during the 2010-2015 was conducting of parasitological research at poultry farms. It was established persistence of temporary and permanent ectoparasites among the poultry. It was studied the epizootic situation at poultry farms. Production facilities, litter, equipments of the poultry houses were examined. It was identified by a microscope the ectoparasites' species. It was found that existence of the poultry's ectoparasites is a very big problem at small farms especially where, multi-age poultries are contained together and where is used floor keeping. The Mallophagoses which we identified from chickens were *Menopon gallinae*, *Menacanthus stramineus*, *Menacanthus cornutus*, *Goniocotes hologaster*, from turkeys – *Menopon gallinae*, *Menacanthus stramineus*, from ducks and geese – *Anaticola crassicornis*, from ostriches – *Struthiolipeurus struthionis*. It was established persistence of darkling beetles *Alphitobius diaperinus* from broilers and laying hens with floor keeping. Sporadically it was detected colony of red chicken's mite *Dermanyssus gallinae*. At the poultry farms where industrial technology is using the big problem is the red mite *Dermanyssus gallinae*. Mite's populations were detected in farms with ovigerous birds' crosses, quails', waterfowls' breeding. It was established strong tendency to increase of *Dermanyssus gallinae* at the industrial poultry sector of Ukraine. The problem with dermanissioz in Ukrainians' poultry is still going and requiring immediate decision.

Keywords: red chicken's mite, mallophagoses darkling beetles, ectoparasites

S1-0328 Characterization and evaluation of the potential for select gastrointestinal microbials as probiotics for poultry production

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Antibiotic resistance is a great concern to the poultry industry. Therefore, there is need to minimize excessive use of antibiotics in poultry production. Alternatives such as probiotics have been evaluated as potential antimicrobials. The objective of this study was to identify and characterize gastrointestinal flora with the potential to serve as probiotics in poultry production. A metagenomics library of the chicken gastrointestinal tract (GIT) was developed targeting the bacterial hypervariable regions (V1-V9) encoding 16s rRNA genes. The region is diverse and can distinguish GIT bacterial populations. Select microorganisms were evaluated for their ability to tolerate and survive GIT pH and bile concentrations, attach to GIT mucosa, and inhibit growth of pathogenic microorganisms. Microbes' survival at pH 7, 6, 5, 4, 3 and 2 for 5hrs, and bile concentrations of 3, 2, 1.5, and 1% for 6hrs, and ability to sequester cholesterol was evaluated. Scanning electron microscopy was used to assess microbial attachment to intestinal mucosal and agar spot test assessed inhibition of growth of pathogenic bacteria. Among the bacteria communities identified were *Lactobacillus reuteri*, *L. plantarum* and *Bifidobacterium longum*. *B. longum* and *L. plantarum* grew well ($P < 0.05$) at pH range of 5 - 7 and 4 - 7, respectively, and inhibited growth of pathogenic microbes such as *E. coli*, salmonella and campylobacter. *L. plantarum* had much higher colony forming units per ml within each pH level, except at pH 2. Both *L. plantarum* and *B. longum* tolerated the various bile concentrations and showed remarkable ability to attach to the surface of the GI tract with obvious changes in the cell surface and cytoskeleton where they were anchored. *L. reuteri* and *B. longum* showed the potential to reduce cholesterol directly or indirectly through production of levan. Overall, the results of this project suggest that these microbes have a strong potential for use as probiotics in the poultry industry.

Keywords: poultry, probiotics, metagenomics, direct fed microbials, bacteria

S1-0329 Flavin containing monooxygenase 3 gene variation modulates the lipid metabolism response to dietary Canola meal supplementation in laying hens

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Hepatic flavin- containing monooxygenase 3 (FMO3), responsible for trimethylamine- oxygenation and fishy egg tainting, has been found to exert broad effects in modulating glucose and lipid homeostasis. To explore the FMO3 genetic variants-associated metabolic patterns in birds provided with the defined diets that contain different amounts of trimethylamine precursor, we provided hens (AA and TT) genotyped at FMO3 c.984 A>T with the corn-soybean meal (SM) basal diet or the basal diet supplemented with 21% of canola meal (CM), which contains higher levels of trimethylamine precursor. The metabolic and hepatic transcriptional analysis revealed a prominent change in lipid metabolism associated with the genetic variation in FMO3 in a diet-specific manner. On the SM diets, the plasma of TT genotype hens predominantly demonstrate higher levels of lipids and acetone, and lower levels of glucose and TMAO than that of AA genotype hens. By contrast, TT genotype hens is characterized by higher levels of glucose but lower levels of lipids when fed the CM diets. The hepatic transcriptional profiling also links the genetic variation in FMO3 to lipid metabolism, and the change of gene expression exhibits similar diet-specific patterns to the metabolic changes, including biosynthesis of long chain polyunsaturated fatty acids, synthesis and transporting of triglyceride and cholesterol, primary bile acid biosynthesis. The reciprocal changes in lipid metabolism induced by this mutation under the SM and CM diets may be partially mediated through the liver X receptor pathway and polyunsaturated fatty acids metabolism. These data suggest that FMO3 is a novel mediator of hepatic lipid metabolism in laying hens, and reveal the dependent of the FMO3 regulatory role on the dietary trimethylamine precursor levels. Our results further support a possible crosstalk between the xenobiotic metabolism and lipid metabolism.

Keywords: FMO3, lipid metabolism, canola meal, laying hens, trimethylamine

S1- 0330 Effects of schizochytrium oil on performance, plasma lipid metabolism and immune function during the peak production of laying hens

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The current study was designed to investigate the effects of diets added different levels of Schizochytrium oil (SO) on performance, egg quality, plasma protein metabolism, lipid metabolism and immune function of the laying hens at peak production. (Method) A total of 288 Jing Hong laying hens aged 28-week-old with similar laying rate were randomly divided into 4 dietary treatments with different inclusion level of SO (0, 0.25%, 0.50% or 1.00%). Each treatment contained 6 replicates with 12 hens each. The experiment lasted for 28 days. (Result) The results showed as follows: 1) compared with the control group, dietary SO supplemental level had no significant effects on egg performance, egg quality and egg component ($P > 0.05$). 2) The 0.50% and 1.00% SO groups significantly increased the Globulin content and decreased total cholesterol, triglyceride and low density lipoprotein-cholesterol in plasma compared to control group ($P < 0.05$). 3) The contents of Immunoglobulin G, M and A in plasma in 0.25, 0.5 and 1% SO fed groups were significantly higher than those in control group ($P < 0.05$), and reduced Prostaglandin E2 (PGE2) in spleen was observed in SO fed chicks than the control ($P = 0.08$). (Conclusion) These results indicate that diet supplement 1.00% Schizochytrium oil can improve plasma lipid metabolism, antioxidant capacity and immune function of laying hens without reduced the egg performance, egg quality and egg components during the laying peak period.

Keywords: schizochytrium oil, laying hens, lipid metabolism, antioxidant capacity, immune function

S1-0331 Effect of feeding guanidinoacetic acid and L- arginine on immune response in aged broiler breeder hens

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Two experiments were conducted to evaluate the effects of feeding guanidinoacetic acid (GAA) and L- arginine (L-Arg) on immune response of aged broiler breeder hens. In the first experiment; a total of 200 broiler breeder hens (Ross 308), 53 weeks (wks) of age were used, and randomly distributed into four dietary treatments (0, 0.6, 1.2 and 1.8 g GAA/kg) each in five replicates. In the second experiment; 320 broiler breeder hens (Ross 308) in a 2×4 factorial arrangement (0 or 1.2 g GAA/kg and 0, 3, 6, and 9 g added Arg/kg diet) were used from 53 to 62 wks of age. Hens received a diet that contained 2800 kcal ME/kg and 14% CP. Indices of humoral immunity [anti-sheep red blood cell (SRBC) titer, IgG, and IgM at 61wk of age] as well as differential leukocyte numbers (62 wk of age) were measured. Supplementary L- Arg increased total anti-SRBC titer ($P \leq 0.048$). However, the effect of supplementary L-Arg on IgM, IgG level, and differential leukocyte numbers was not significant. In second experiment, dietary GAA increased IgM level ($P \leq 0.029$). The effect of the GAA (in two experiments) on the total anti-SRBC titer, IgG level and differential leukocyte numbers was not significant. The interactive effects of L-Arg and GAA on the total anti-SRBC titer, IgM, IgG and differential leukocyte numbers were not significant. In conclusion, it seems that L-Arg and GAA supplemented diet can improve humoral immunity of broiler breeder hens.

Keywords: arginine, broiler breeder, guanidinoacetic acid, immunity, leukocyte

S1-0332 Effect of mycotoxins in the diet on intestinal integrity and performance of broiler chicks in pre-starter phase

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This experimental study was conducted to evaluate the effect of corn with high levels of mycotoxins (CHMT): Aflatoxins (AF: 11.5ppb), Ochratoxin A (OA: 5.6ppb), deoxynivalenol (DON: 1.2ppm) and fumonisin (FU: 35ppm) in the diet on the relative weight of the gut and organs, performance and profit of chicks from 0-7 days old. 45 chicks Cobb 500 male of one-day-old, under restriction of water and food, were used and were divided into three treatments based on a basal diet (BD) with two types of corn: low level (CLMT) and high level (CHMT) of mycotoxins, which were: 1. BD + CLMT; 2. BD + CHMT and 3. BD + CHMT + natural binder, with three repetitions each, in a randomized complete block design (RBD). The relative weights of the duodenum, jejunum, ileum, cecal, liver, heart, spleen and Bursa, weight gain, feed intake, feed conversion, energy efficiency and profit were evaluated. Data were analyzed using the GLM procedure of SAS (2003). The CHMT of AF, OA, DON and FU in the diet did not influence ($P > 0.05$) the relative weight of intestinal segments and organs, weight gain, feed conversion and energy efficiency. The CHMT increased ($P < 0.05$) the relative weight of Bursa and feed intake, and decreased 6.4% the profit. It is concluded that under the study conditions, the chicks have a relative physiological capacity to tolerate the assessed levels of CHMT in the diet.

Keywords: corn, mycotoxins, binder, chickens, diet

S1-0333 Effect of dietary plant extract derived from *Dracontium lorentense* on performance of broilers chicks in the initial phase

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This study was conducted to assess the effect of dietary plant extract derived from *Dracontium lorentense* on performance of chicks from 0-14 days-old, and to determine whether it could have the potential as alternative natural growth promoter to antibiotics. One hundred and forty four, 1-day-old male broiler chicks (Cobb 500) were randomly assigned to three experimental groups as treatments and four replicates each in a randomized complete block design. The groups were: 1. Basal diet without antibiotic, 2. Basal diet + 50 mg Zinc Bacitracin/Kg diet, 3. Basal diet + 10 g of extract *Dracontium lorentense*/Kg diet. Each group was fed ad libitum. The characteristics of body weight gain, feed intake, feed conversion, energy efficiency, protein efficiency, mortality and profit were evaluated. The data were analyzed using the GLM procedure of SAS (2003). Plant extract derived from *Dracontium lorentense* not affect ($P > 0.05$) body weight gain, feed conversion, energy efficiency and protein efficiency; however, the group of chicks fed with the plant extract derived from *Dracontium lorentense* achieved numerical differences higher of 28 g (7.1%) in body weight gain, compared to group with antibiotic. Feed intake was lower ($P < 0.01$) in group with *lorentense* *Dracontium* extract. The profit was best for the group of chicks with *lorentense* *Dracontium* extract in 4.38%. The group with antibiotic had 4.16% mortality. It was concluded that the dietary plant extract derived from *Dracontium lorentense* maintains performance and improved profitability, and may be considered a potential natural growth promoter to replace antibiotics in the diet of chicks in the initial phase.

Keywords: broiler chicks, plant extract, antibiotic, performance.

S1-0334 Effects of dietary oregano oil supplementation on performance parameters and intestinal morphology of Japanese quails (*coturnix coturnix japonica*) reared under normal and cold temperatures

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The aim of this study was to assess the effects of dietary oregano oil supplementation (Ropadiar®) on growth characteristics in Japanese quails (*coturnix coturnix japonica*) reared in normal and cold temperatures. Three hundred 1-day old Japanese quails randomly divided into five groups (One control group and two treatment groups of 100 and 200 mg of essential oil of oregano/kg in normal temperature (groups I, II and III); One control and a treatment group of 100 mg Oregano essential oil/kg in cold stress situation (groups VI and V). All the groups were subjected to the same temperature regimen until 14th day. At the age of 14, the cold stress groups were transferred into a different house in which the temperature was ranged between 10 to 13 °C until 35th day of age. At the end of the experiment, performance parameters and intestinal morphology was analyzed, performance parameters were not affected by supplementing oregano oil in normal temperature. On the other hand, dietary supplementation of oregano essential oil at the level of 100 mg/kg under cold stress situation improved Japanese quail's body weight, FCR and intestinal villus morphology. This study indirectly states that the supplementation of the oregano essential oil components to Japanese quail's diet would delay the adverse effects caused by cold stress on the birds performance.

Keywords: oregano oil supplementation, Ropadiar®, Japanese Quail, cold stress

S1-0336 Effects of dietary octacosanol supplementation on growth performance, carcass characteristics and meat quality of broiler chicks

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Octacosanol, with prominent physiological activities and functions, has been recognized as a potential growth promoter in animals. The objective of this study was therefore, to test the efficacy of dietary octacosanol supplementation on growth performance, carcass characteristics and meat quality in broiler chicks. A total of 392 one-day-old male Arbor Acres (AA) broiler chicks with similar body weight were randomly distributed into 4 dietary groups of 7 replicates with 14 birds each supplemented with 0, 12, 24 and 36 mg octacosanol (extracted from rice bran, purity >92%)/kg feed. The feeding trial lasted for 6 weeks. The results showed that broilers fed a diet containing 24 mg/kg octacosanol significantly improved growth performance compared with those fed the control diet in the overall phase (d 1 to 42, $P=0.042$). Dietary with 24 or 36 mg/kg octacosanol supplementation significantly increased eviscerated yield by 5.88% and 4.26% respectively, than those fed the control diet ($P=0.030$). Breast muscle yield of broilers fed with 24 mg/kg octacosanol diet was increased by 12.15% compared with that of control diet ($P=0.047$). Broilers fed with 24 or 36 mg/kg octacosanol diet had greater pH_{45min} value in the breast muscle ($P=0.021$), and great lower drip loss value ($P=0.007$) between the octacosanol-added and the control groups. These studies indicate that octacosanol is a potentially effective and safe feed additive which may apparently improve feed efficiency and meat quality, stimulate eviscerated and breast muscle yield in broiler chicks.

Keywords: octacosanol, growth performance, carcass characteristics, meat quality, broiler chicks

S1-0337 Octacosanol plays a regulatory role in promoting the reproductive performance of laying hens

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This experiment was conducted to evaluate the effects of dietary supplementation octacosanol, extracted from rice bran, on laying performance, egg quality, serum hormone level and reproductive gene expression in laying hens. A total of 360 Hy-line Brown (67-week old) laying hens were randomly assigned to 1 of 3 treatments with 0, 5 and 10 mg octacosanol (purity>92%)/kg feed. The trial lasted for 10 weeks. The results showed during the overall phase, hens fed with 5 and 10 mg/kg octacosanol diet showed lower feed conversion, which was greatly significantly decreased by 4.88% and 3.41% ($P<0.01$), compared with those fed the control diet. At the end of 77 week, in comparison to the control group, dietary supplementation with 5 and 10 mg/kg octacosanol greatly significantly increased the albumen height by 20.55% and 13.31% ($P<0.01$), respectively, and HU by 12.94% and 8.70% ($P<0.01$). The eggshell strength had greatly increased by 39.48% and 24.50%, respectively ($P<0.01$). Through comparing the serum hormone levels and mRNA expressions related to reproductive axis with the control, we found dietary octacosanol supplementation could significantly increased the concentrations of serum T3, E2, P4, FSH and LH ($P<0.05$) and up-regulated the mRNA expression level of FSHR, LHR and PRLR in preovulatory follicles, small yellow follicles and large white follicles. Our observation provide an evidence that octacosanol has the function of improving reproductive performance and reveal the action mechanism, which indicate octacosanol is a potentially effective and safe feed additive in laying hens production.

Keywords: octacosanol, laying performance, egg quality, hormone level, mRNA expression

S1- 0338 Reducing lipid accumulation by 5-HTP in broilers

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The present study was conducted to explore the effects of 5-hydroxytryptophan (5-HTP) on the regulation of lipid metabolism-associated genes and immunological factors in broilers. Two hundred AA broilers were randomly divided into two groups: one received 0.2% 5-HTP in the basic diets and the other received the basic diets (Control). Treatment of broilers with 5-hydroxytryptophan (5-HTP) stimulated an decrease ($P<0.05$) in the abdominal fat weight and the ratio. 5-HTP treatment had no significant effect ($P>0.05$) on the plasma concentration of triglyceride (TG), non-esterified fatty acid (NEFA), 5-hydroxytryptamine (5-HT), very low density lipoprotein (VLDL) and adiponectin (ADP) when compared with control broilers. However, the expression of tryptophan hydroxylase 1 (TPH1) was significantly ($P<0.05$) increased in the duodenum and ileum while the mRNA levels of 5-hydroxytryptamine receptor 2A (5-HT2A) was no different in the liver and fat in 5-HTP-treated broilers. Treatment of broilers with 5-HTP had no significant ($P>0.05$) effect on the mRNA levels of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), carnitine palmitoyl transferase 1 (CPT1) and peroxisome proliferator-activated receptors (PPAR) in the liver and the abdominal fat. However, the mRNA levels of adiponectin receptor 1 (ADP1R) and adiponectin receptor 2 (ADP2R) in the fat had a tendency to increase by 5-HTP treatment, but there is no different on western blot. Moreover, the expression of interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor (TNF) had a tendency to increase in the fat. In conclusion, these results suggest that 5-HTP plays a unique role in lipid metabolism.

Keywords: lipid metabolism; immune; 5-HTP; broilers

S1- 0339 Effects of wheat particle size and feed form on the growth performance and gastrointestinal tract traits of broilers

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This study is conducted to evaluate the effects of wheat particle size (2, 4, 6, 8 and 10mm) and feed form (mash and pellet) on the growth performance and gastrointestinal tract traits of broilers aged from 1 to 42 d. 770 1-d-old male broilers (Ross308) randomly assigned to 10 dietary treatments with 7 replicate of 11 broilers each. Broilers fed on the mash diets showed better development of digestive tract but worse growth performance. A linear increase and quadratic response in feed per gain over the course was observed as the wheat particle size increased in both feed form. In the mash diets, a linear improvement and quadratic effect were observed at 21 d for the development of the gizzard and the duodenum. In the pellet diets, a quadratic effect was observed on the performance of the starter phase as the wheat particle size increased. An interaction between feed form and particle size was observed on the relative weight of the 21d gizzard, 42d liver and morphology of the jejunum. The broilers performed better when fed the pellet diets. Coarsely ground wheat and mash diets had a stimulating effect on the development of the gastrointestinal organs.

Keywords: broiler, gizzard, mash, particle size, pellet, wheat

S1-0340 Evaluation of Moringa oleifera leaf in laying hens: Effects on laying performance, serum biochemical and organ histopathological indices

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An experiment was conducted to evaluate the effects of Moringa Oleifera leaf (MOL) on performance, egg quality and health of laying hens. A total of 360 27-week-old Hy-Line Grey commercial layers were randomly allocated into one of the four groups. The control group was fed a corn-soybean meal basal diet, and other groups were fed basal diets supplemented with 5%, 10% or 15% of MOL. The experiment lasted for 8 weeks. The results showed that no significant difference was observed on egg weight or feed intake among all groups. Laying hens fed diets containing 5% or 10% MOL had no significant difference in egg production compared with the control group, while egg production was decreased when birds fed diets containing 15% MOL. An increase was observed on albumen height and Haugh unit when birds fed 5% and 10% MOL. There was an increase on yolk color when birds fed the diets containing MOL. A significant increase was observed on plasma total protein when birds fed diets containing 5% MOL. Diets containing 15% of MOL increased plasma aspartate aminotransferase activities, and levels of uric acid and albumen. It showed that laying hens fed 5% and 10% supplementation of MOL did not induce histological lesions in the livers and kidneys, while birds fed 15% MOL induce histological lesions. In conclusion, our observations suggest that dietary supplementation of MOL ($\leq 10\%$) could be added into hens' diets. Dietary MOL supplementation of 5% was recommended evidenced by the performance and biochemical and histopathological indices.

Keywords: Moringa oleifera leaf, laying performance, egg quality, laying hens, serum biochemical, organ histopathological

S1- 0341 Effect of dietary protein sources on organism health of laying hens and albumen quality under refrigerated conditions

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The shortage of soybean meal has been a limiting factor to poultry production, and becoming increasingly challenging. Exploitation and evaluation of non-traditional ingredients is of great importance for sustainability of laying hen industry. The objective of this study was to investigate the effects of dietary protein sources on organ index, liver parameters, histochemistry of laying hens, and albumen quality under refrigerated conditions. Two hundred and sixteen laying birds of 32 weeks of age were allotted into 3 treatments groups with 6 replicates of 12 birds each. The control group was fed corn-soybean meal basal diet (SBM), and 2 of the experimental diets included 195 g/kg low-gossypol cottonseed meal (LCSM) or 292 g/kg double-zero rapeseed meal (DRM), which corresponded to 100% (LCSM100), and 100% (DRM100), respectively, of the SBM protein replaced by LCSM or DRM. The experimental diets, which were isocaloric (ME, 11.11 MJ/kg) and isonitrogenous (CP, 16.5%), had similar digestible amino acid profiles. The feeding trial lasted for 12 wks. The results showed as follows: 1) Compared to SBM, LCSM100 decreased oviduct index at the end of 12 w ($P < 0.05$). The abdominal fat rate and liver fat rate were lower in DRM100 than in SBM at the end of the trial ($P < 0.05$). 2) The villous height of jejunum in LCSM100 group was lower than in the other treatments ($P < 0.05$). The mucosal columnar epithelial cell layer of magnum in LCSM100 group was incomplete, mild swelling of tubular glands, mainly fold almost cannot see gland secretions. 3) Compared with SBM, LCSM100 and DRM100 significantly ovomucin yield when eggs stored 28 d at 4°C. It was concluded that LCSM100 as sole protein source in laying hens diets on the jejunum absorption ability and magnum secretory capacity have a negative effect. LCSM100 or DRM100 negatively affected albumen quality under refrigerated conditions.

Keywords: laying hens, dietary protein sources, organism health, albumen quality

S1- 0342 Growth response, serum biochemistry and organ histopathology of broilers fed diets supplemented with graded levels of petiveria alliaca root meal

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An experiment was conducted with 180 unsexed day old broiler chickens to assess the growth rate, serum biochemistry and organ histopathology of broiler chickens fed diets supplemented with graded levels of Petiveria Alliacae root meal (PRM) of 0, 500, 1000, 1500, 2000 and 2500g/100kg of feed in a 8 weeks feeding trial. Data were collected on feed intake and weekly weight gain. Blood samples were collected from the animals through the wing web vein for serum biochemistry while samples of visceral organs and small intestine were collected from the birds after they were stunned and sacrificed after 8 weeks feeding trials. Results showed that there were no significant difference ($P > 0.05$) in all the growth response parameters examined. The serum proteins and Alanine amino transferase examined were not significantly ($P > 0.05$) affected by the dietary treatment. Aspartate amino transferase (AST) of the experimental birds was significantly higher in broilers fed 0, 500, 1000 and 1500g PRM than others. Birds fed 2500g PRM recorded the highest value for Glucose and Cholesterol, while birds fed 1500g PRM/100kg of feed recorded the lowest value for these parameters. Relative organ weight of various visceral organs examined apart from lung, kidney and spleen were significantly influenced by the dietary treatments. The results of histopathological examination revealed damages done by dietary treatment. Necrosis of the villi and tubular epithelium were observed in the intestine and kidney in all the treatment except the control. The liver of the birds fed PRM showed signs of hepatocellular necrosis and increased mononuclear/inflammatory cells in the hepatocyte. These conditions increased as the inclusion of PRM increased. The results suggest that although PRM possess good phytoantic properties for optimal growth of the broilers, inclusion levels above 1500g/100kg of feed in the diet of broilers induced severe necrosis of the villi and hepatocellular necrosis in the liver.

Keywords: petiveria alliaca, broilers, growth response, serum, histopathology, organ weight

S1-0343 Effects of different oilseed cakes supplementation in laying quails diet on laying performances, egg quality characteristics, serum biochemical parameters and fatty acid compositions of egg yolk

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The aim of this experiment was to evaluate the effects of different oilseed cakes supplementation in laying quails diet. A total of 240 laying quails (13-week-old) were randomly assigned to 3 dietary treatments with 4 replicates. Laying quails were fed corn and soybean meal-based diets containing 0%, 5% rapeseed cake (RSC) and 5% linseed cake (LSC) for 35 days, respectively. The results showed that the supplement of RSC and LSC had no adverse effects on laying rate, average feed intake, egg shape index and yolk weight percentage ($P > 0.05$). Average egg weight, eggshell weight and eggshell thickness were significant decreased while feed/egg ratio and broken egg rate increased significantly on RSC and LSC groups ($P < 0.05$). The supplementation of RSC and LSC did not affect the serum concentration of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), globulin (GLB), free triiodothyronine (FT3), free thyroxine (FT4), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total antioxidant capacity (TAC) and superoxide dismutase (SOD) ($P > 0.05$). However, malondialdehyde (MDA) level in serum of LSC group was significantly higher compared with control group ($P < 0.05$) but similar to RSC group ($P > 0.05$). Both LSC and RSC increased the concentration of linoleic acid, α -linolenic acid, eicosapentaenoic acid (EPA) and total polyunsaturated fatty acid (PUFA) ($P < 0.05$) but the concentration of monounsaturated fatty acid (MUFA) in egg yolk decreased significantly ($P < 0.05$). In addition, LSM also significantly increased the concentration of docosahexaenoic acid (DHA) ($P < 0.05$). In conclusion, 5% RSM and LSM supplement had no significant effect on laying quail health and improved the proportion of PUFA, but to some extent affected egg quality characteristics. These results provide a reference for rapeseed and linseed cakes utilization on laying quails.

Keywords: laying quails; rapeseed cake; linseed cake; egg quality; fatty acid

S1-0344 Impact of fumonisins in layers and effect of a counteracting strategy

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Fumonisin (FUM) interact with birds' immune and digestive system. To prove the effectiveness of FUM counteracting strategies in vivo, suitable biomarkers have to be used. One counteracting strategy is the enzymatic degradation of FUM by a novel feed additive (FUMzyme[®]). This enzyme converts FUM via the partially hydrolysed forms (pHFBs) to the non-toxic, fully hydrolysed forms (HFBs). The aim of this study was to evaluate the effect of fumonisins and FUMzyme[®] on general performance, egg production and on the degradation products of fumonisins in excreta (biomarker of exposure) of laying hens. A total of 120 laying hens (Lohmann Brown, 20 weeks old) were randomly assigned to 30 pens with 4 layers/pen. Three groups were investigated with 10 replicates each: Group 1 was the negative control, group 2 the positive control with 10 ppm FUM and group 3 the product group with 10 ppm FUM and 15 Units FUMzyme[®] per kg finished feed. Parameters evaluated were performance (live weight and weight gain, feed consumption and conversion rate), egg parameters (egg number and weight) and FUM biomarkers of exposure. The trial lasted for 14 days. Feed was analyzed for mycotoxin contamination via HPLC method. Statistical analysis was done with ANOVA (IBM SPSS 19.0). 10 ppm of FUM had no significant negative effect on performance parameters (feed intake, body weight and weight gain). A negative impact on egg mass and laying rate as well as on FCR was observed in groups fed FUM contaminated feed compared to control and additive group. FUMzyme[®] showed a significant ($P < 0.05$) reduction of FB1 and an increase of HFB1 in excreta compared to the toxin control group. Though no negative impact of FUM on general performance could be observed within the two-week trial period, the negative trend on egg performance was obvious and counteracted by the addition of FUMzyme[®]. Furthermore, the effectiveness of the additive was clearly proven by analysis of FUM biomarkers of exposure.

Keywords: mycotoxin, fumonisins, mycotoxin deactivation (FUMzyme[®]), feed additive, biomarker

S1-0346 Yeast mannan oligosaccharide and/or nucleotides from different sources in broilers diet

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This study evaluated the effects of supplementation of each of 4 yeast mannan oligosaccharide and/or nucleotide sources on broiler performance. For this, 3,000 one-day-old Cobb male chicks were distributed in a completely randomized block design, with 6 treatments and 10 blocks with 6 reps (0.29 m²/bird). The treatments were: T1- Control diet (no additive supplemented); T2- Diet with Bacitracin Methylene Disalicylate [BMD] (55 g/MT, from 0-42 d); T3 - Diet with yeast cell wall from *Saccharomyces cerevisiae* (0.5 kg/MT of product named ImmunoWall® [YCW], from 0-42 d); T4- Diet with yeast cell wall from *Saccharomyces cerevisiae* (0.5 kg/MT of commercial product named MOS1 from 0-42 d); T5- Diet with inactive yeast cells from *Pichia guilliermondii* (0.5 kg/MT of product named MOS2, from 0-42 d); T6- Diet with nucleotide source from *Saccharomyces cerevisiae* (1 kg/MT from 0-14 d and 0.5 kg/MT from 14-42 d using product named Hilyses® [YNU]). Built-up litter was used. Study criteria included body weight gain (BWG, kg), feed intake (FI, kg), feed/gain ratio (FGR), and mortality rate (%) at 28 and 42 d. The data were analyzed using the SAS LSD test ($P \leq 0.05$) to separate means when ANOVA F values are significant ($P \leq 0.05$). The birds supplemented with BMD had greater FI (+5.2%) when compared to MOS2 ($P < 0.05$) at 28 d. For BWG, birds fed diets with BMD (+4.8%) and YCW (+4.0%) showed the best results when compared to MOS2 ($P < 0.05$). However, the birds treated with YNU had best FGR (-2.0%) compared to control ($P < 0.05$) at 28 d. The treatments had no effect ($P > 0.05$) on FI, BWG, or mortality rate at 42 d. However, diets supplemented with BMD (-2.1%) or MOS2 (-2.8%) had improved ($P < 0.05$) FGR compared to control birds whereas birds treated with YNU showed best FGR (-3.2%) when compared to control and MOS1. In summary, this study demonstrated that broiler diets supplemented with BMD, MOS2, or YNU significantly improved feed/gain ratio from 0-42 d on built-up litter compared to control diets.

Keywords: bacitracin, prebiotic, *saccharomyces cerevisiae*, performance

S1-0347 Emulsifier on broilers diets with energy reduction and different fat sources

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This study evaluated the effects of emulsifier supplementation on broilers diets with energy reduction and two fat sources over performance. For this, 1,200 one-day-old Cobb male chicks were distributed in a completely randomized design with a factorial arrangement 2x2x2 + 2 (2 fat sources; 2 metabolizable energy (ME) levels; with emulsifier or not) + control diets with 2 fat sources, totaling 10 treatments, with 10 reps of 12 birds each (12 birds/m²). The treatments were: T1- Control diet with degummed soybean oil (CS); T2- Control diet with acid oil (CA); T3 - T1 with reduction of 80 kcal of ME (CS80); T4- T1 with reduction of 100 kcal of ME (CS100); T5- T2 with reduction of 80 kcal of ME (CA80); T6- T2 with reduction of 100 kcal of ME (CA100); T7- T3 + emulsifier (L) (CS80L); T8- T4 + L (CS100L); T9- T5 + L (CA80L); T10- T6 + L (CA100L). The emulsifier was included in diets at 0.5 kg/MT (product named Liposorb®). Study criteria included body weight gain (BWG, kg), feed intake (FI, kg), feed/gain ratio (FGR), viability (V, %), and production factor ($PF = [BWG * V / FGR * age] / 100$) at 42 d. The data were analyzed using the SAS F test for orthogonal contrasts ($P \leq 0.05$). The birds supplemented with L and with 80 kcal reduction and soybean oil had same ($P > 0.05$) performance when compared to CS. However, when it was reduced 100 kcal, the L supplementation was not able to achieve the same ($P < 0.05$) FGR and PF compared to CS. Although, CS100L improved ($P < 0.05$) BWG and FP when compared to CS100. For acid oil fat source, CA80L improved ($P < 0.05$) FI, but had no effect ($P < 0.05$) on the other parameters, compared to CA. However, when it was reduced 100 kcal, the L supplementation was not able to achieve the same ($P < 0.05$) FI and FGR compared to CA. CA100L improved ($P < 0.05$) V and FP when compared to CS100. In summary, this study demonstrated that broiler diets supplemented with L did not affect BWG and FGR when it was reduced 80 kcal/kg of ME compared to control diets from both fat sources.

Keywords: performance, acid oil, soybean oil

S1-0349 The effectivity of a multi enzyme on the performances of laying hens fed with different ingredients

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An experiment was carried out to evaluate the effectivity of a multi enzyme produced by *Eupenicillium javanicum* BS4 on the performance of laying hens fed with different feed ingredients. Three diets were formulated with the same nutrients (crude protein, ME, digestible- lysine, methionine + cystine, tryptophan, Ca and av. P) value to meet the nutrient requirement of laying hens. Diets were formulated either based on corn, palm kernel cake or rice bran. The diets were either supplemented or not supplemented with the enzyme (1.6 l/ton feed) and fed to Isa Brown pullet from 16 to 38 weeks old. Each diet was fed to 24 birds (6 replicates, 4 birds/replicate) and the performance were recorded for the last 18 weeks. The egg quality (egg shell thickness and yolk color score) were measured at 27 weeks of age. Data were analysed statistically with 2 (enzymes) X 3 (feedstuffs) factorial design, followed by Duncan's multiple range test when the analyses of variance was significant ($P < 0.05$). Results of the experiment showed that the interaction effect between enzyme supplementation (E) and kind of feedstuffs (F) was only significant ($P < 0.01$) on yolk color. The yolk color was improved by enzyme supplementation only when the PKC was included in the diet. The enzyme supplementation significantly increased the HD egg production ($P < 0.05$) and the FCR ($P < 0.01$) but not the egg weight, feed intake nor the egg shell thickness. Different kind of feedstuffs significantly ($P < 0.01$) affect the HD egg production, feed intake and egg shell thickness. Birds fed with palm kernel cake diet consumed more feed and produce more eggs than those fed with corn-soy or rice bran diet. Birds fed with rice bran diet have a significantly thicker egg shell than birds with other diets. It is concluded that supplementation of enzyme produced by *E. javanicum* BS4 into diets (based on corn-soy, palm kernel cake or rice bran) was effective to improve egg production and feed conversion ratio of laying hens.

Keywords: enzyme, palm kernel cake, rice bran, egg production

S1-0350 Effects of dietary processed sweet potato waste products on the growth of broiler chicken

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In Japan, there is a big amount of sweet potato waste products (unused pieces mainly composed of peel of tuberous root) generated in the process of making shochu. This study was conducted to evaluate the effects of processed sweet potato waste products on the growth performance, digestibility of protein, energy utilization, and antioxidant of broiler chicken. A total of 24 chicks of similar body weight (380 ± 16 g) were divided into 3 groups (CTRL: control group; ATDSP: air temperature dried sweet potato waste; HTDSP: high temperature dried sweet potato waste) and fed with experimental diets for 14 days. The sweet potato waste were about 50% instead of corn in ATDSP group and HTDSP group. The results showed as follows: 1) The feed conversion of HTDSP group was significantly increased compared with ATDSP group ($P < 0.05$), while there was no significant difference of the body weight gain and feed intake among three groups. 2) The abdominal fat ratio and muscular fat content of HTDSP group were significantly higher than that of ATPSP group ($P < 0.05$), while there was no significant difference between HTDSP group and CTRL group. And the plasma triglyceride content of three groups showed no significant difference. 3) Both crude protein digestibility and energy utilization of HTDSP group were significantly higher than that of ATDSP group ($P < 0.01$), but significantly lower than that of CTRL group ($P < 0.05$). 4) The plasma α -tocopherol concentration of two sweet potato groups were very significantly higher than the CTRL group ($P < 0.01$), and in both plasma and breast muscle TBARS of two sweet potato groups were significantly lower than the CTRL group ($P < 0.05$). In conclusion, the high temperature dried sweet potato waste products achieved the same growth performance as corn meal for broilers and it even had potential to improve the meat quality of broilers.

Keywords: sweet potato waste, broiler, growth, antioxidant

S1- 0351 1, 25- calcitriol- glycoside (panbonis®) enhances growth performance, bone mineralization and immune status of broilers fed diets containing low available phosphorus

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Vitamin D designates a group of closely related compounds and is as such biologically not active; it requires two metabolic conversions to the active metabolite 1,25-dihydroxyvitamin D3 (1, 25(OH)2D3). To study the effectiveness of Panbonis® when supplemented to diets (isocaloric and isonitrogenous) containing low available phosphorus (avP), 1,400 one-day-old male broiler chicks (Ross 308) were divided into 7 dietary treatments (10 replications). The diets were a positive control (PC, 0.5 and 0.45% avP in starter and grower diets), moderate avP reduction (0.3 and 0.25% avP), and high avP reduction (0.2 and 0.15% avP) in combination with 100 and 200 g/ton of Panbonis®. Ca:avP ratio in all diets was maintained at 2:1. Growth performances of broilers decreased with a reduction of avP ($P < 0.05$). The supplementation, Panbonis® improved broiler performances at both levels of avP reduction to the similar level of PC group ($P < 0.05$). The supplementation of Panbonis® showed a superior effect on growth performances ($P < 0.05$). Bone breaking strength and bone mineralization were improved with Panbonis® supplementation ($P < 0.05$). A significant reduction in IgG ($P < 0.05$) was observed in birds fed diets with Panbonis®. The supplementation of Panbonis® therefore, elicits better broiler performances, bone mineralization and immune status.

Keywords: broilers, 1, 25 calcitriol- glycoside, bone mineralization, tibia ash, immune status

S1-0352 L-methionine, DL- methionine and DL-hydroxy methionine are equivalent to sustain broiler growth performance

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The current trial was conducted to compare bird's performance in response to diets supplemented with L- Methionine, DL- Methionine and DL- Hydroxy Methionine (DL- HMTBA). 1050 day- old Ross PM3 chicks were allocated in 10 treatments with 7 replicates each. Diets were formulated according to Ross recommendations and consisted of a basal diet without methionine addition and 9 treatments supplemented with three graded levels of either DL- Met, L- Met or DL- HMTBA. Birds were reared in floor pens from 0 to 42- d, divided in three feeding phases: 0-14 d, 15-28 d and 29-42 d. For each feeding phase, feed intake, body weight gain and feed conversion ratio were calculated and analyzed using an ANCOVA model with the methionine source and the methionine dose as main factors. Body weight gain for the whole period for each methionine source was then analyzed with an exponential model to determine the bio-equivalence between L- Met, DL- Met and DL- HMTBA. During the starter phase (d0-14), L- Met gave similar growth performance as well as feed conversion ratio in comparison to DL- Met and DL- HMTBA ($P > 0.10$). No difference of feed intake was observed between dietary treatments for growing and finisher phases. Body weight gain and feed conversion ratio were significantly improved when the doses of methionine were increased until a plateau is reached ($P < 0.001$). No significant difference of growth performance and feed conversion ratio were observed between L- Met, DL- Met and DL- HMTBA- fed birds for grower and finisher phases. No interaction was found between the methionine dose and source for all periods and parameters ($P > 0.10$). In addition, the exponential models fitted to each methionine source for body weight gain as response to Met+ Cys intake showed that L- Met, DL- Met and DL- HMTBA lead to similar performance. Collectively, the three methionine sources are equivalent to sustain broiler growth performance and no advantage was found with L- Methionine during the starter phase.

Keywords: broiler, DL- methionine, DL- HMTBA, L- methionine, performance

S1-0353 Increasing dietary hydroxy methionine level benefits to feed hygiene and decreases Salmonella counts

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Interest in the use of organic acids in animal nutrition is based on their protective effect in feed against pathogenic bacteria such as Salmonella, Escherichia coli, etc. Two studies were conducted to determine the effect of DL-Hydroxy Methionine (DL-HMTBA), as feed preservative against common pathogens in feed. A pelleted broiler feed formulated with free of extra methionine was mixed with water in a ratio of 1:2 in order to speed-up bacterial growth and restrict the test duration to 6 days. Trial 1 consisted in three treatments: the control treatment, control + 0.5% of DL-HMTBA and control + 2% DL-HMTBA. In trial 2, a microbial contamination was induced by inoculating an initial concentration of 10⁶ CFU/g of Salmonella enterica in the solution of 1/3 feed + 2/3 water. The two experimental treatments of 0.5% and 2% DL-HMTBA were then applied to determine whether DL-HMTBA could effectively against Salmonella. Analyses of the feed were realized at 0, 5, 24 and 72 h and 6 days after storage at ambient temperature (22 °C). Results of trial 1 showed that the growth and proliferation of total coliforms, Escherichia coli and fecal enterococci were inhibited with 0.5% of DL-HMTBA inclusion. A bactericidal effect was also observed on total coliforms, Escherichia coli and fecal enterococci with 2% of DL-HMTBA supplementation. Trial 2 showed that application of 0.5% of DL-HMTBA inhibited the growth of Salmonella and reduced its concentration by 100 times in 3 days whereas at 2% DL-HMTBA, no Salmonella enterica was detected. Altogether, these studies demonstrated that DL-Hydroxy Methionine effectively prohibited the proliferation of pathogens and even killed them at much higher dose in feed.

Keywords: feed, hygiene, DL-HMTBA, Salmonella enterica, Escherichia coli

S1-0354 Use of garlic and green tea as alternative feed additives in broiler diet

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This study was conducted to compare the effect of garlic and green tea as alternative feed additives in broiler diet. Experiment was done for a period of 35 days with a number of 300 day old straight run broiler chicks. Birds were divided into five dietary treatment groups with 4 replications each having 15 birds per replication. The dietary groups were; control (basal diet; no additives), antibiotic (basal diet + antibiotic), garlic (basal diet + 0.25% garlic powder), green tea (basal diet + 0.25% green tea powder) and combined (basal diet + 0.125% garlic powder + 0.125% green tea powder). Results showed that the body weight, body weight gain and total FCR were increased significantly ($P > 0.05$) in the antibiotic, garlic and combined groups compared to the control. Abdominal fat, total cholesterol and LDL were significantly ($P > 0.05$) lower and HDL was significantly ($P > 0.05$) higher in the supplemented groups compared to the control and antibiotic groups. Triglyceride was significantly ($P > 0.05$) lower in the antibiotic, garlic, green tea and combined groups compared to the control group. Combined group had lower ($P > 0.05$) GPT and creatinine value compared to the control group, whereas lower GOT value was in the antibiotic group. Cost of production per kg live broiler was lower in antibiotic and garlic group comparing control, green tea and combined groups. With regards to profit, antibiotic and garlic groups showed higher profitability compared to the other groups. Taken together, the results indicated that addition of garlic and green tea to broiler diet had positive effect on growth performance, lipid profile and profitability and no negative effect on meat yield, bone development, carcass parameter, GOT, GPT and creatinine values. Based on the results of the present study it can be suggested that the garlic and green tea could be potential feed additives in broiler diet.

Keywords: garlic, green tea, feed additive, broiler

S1-0355 Effect of either DL-methionine or DL-methionine hydroxy analogue on the antioxidant defense system in broilers

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Methionine (Met), a precursor for antioxidants like glutathione (GSH), may influence the animal's antioxidant defense, but differently for different dietary Met sources. Therefore, the effect of DL-Met (DLM) and DL-2-hydroxy-4-(methylthio) butyric acid (DL-HMTBA) on the antioxidant defense system in broilers was investigated. 336 one-day old male Cobb 500 broilers were allocated to 48 cages and 7 groups. Wheat-soybean meal-based basal diets with suboptimal concentrations of sulfur-containing amino acids (0.76, 0.64, and 0.58% Met + Cys during days 0-10, 11-21 and 22-35) were fed not supplemented (Control group) or supplemented with Met as DLM or DL-HMTBA at 0.10, 0.25 and 0.40% (equimolar comparison). Body weight and feed intake were recorded after 10, 21 and 35 days. At day 35, samples of liver and jejunum mucosa were collected (n=6 per group) and analyzed for GSH (photometry), tocopherol (HPLC), and mRNA abundance (RT-PCR) and activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The data were analyzed by ANOVA (fixed factors: Met source, Met concentration, interaction). Feed intake and weight gain were lower and feed:gain ratio higher in the Control compared to all Met-supplemented groups, but did not differ between Met sources. However, relative efficacy of DL-HMTBA was only 86% of that of DLM for feed:gain ratio (regression analysis). Activities and mRNA abundance of SOD, CAT and GPx in liver and of SOD and GPx in jejunum were similar in all groups. CAT activity in jejunum was higher in the Control compared to all other groups. Concentrations of tocopherol related to triglycerides in liver and jejunum and of GSH in liver were similar for both Met sources, but increased with increasing dietary Met. Jejunal GSH concentrations were similar in all groups. In conclusion, Met supplementation increased tissue antioxidant concentrations, but the Met source did not influence the antioxidant defense system in healthy broilers.

Keywords: DL-methionine, methionine hydroxy analogue, oxidative stress, antioxidant defense system

S1-0356 Effect of varying levels of dietary methionine sources on the quality and shelf life of fresh broiler meat

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The objective of the study was to investigate the influence of different methionine (Met) sources and concentrations on quality, spoilage and shelf life of broiler chicken meat. In total seven groups were tested: one basal group deficient in Met+Cys (0.76, 0.64, and 0.58% Met + Cys during 0-10, 11-21 and 22-35 days of age) and three doses (0.10, 0.25, and 0.40%) of either DL-Methionine (DLM) or DL-2-hydroxy-4-methylthio butanoic acid (DL-HMTBA) on an equimolar basis. Chickens were fed the experimental diets for 35 days. After slaughter, fillets were aerobically packed and stored under temperature-controlled conditions at 4°C. Meat quality investigations comprised microbial (total viable count, *Pseudomonas* spp.) physicochemical (pH-value, drip loss) and sensory parameters. For the determination of quality loss, a ranking test with the parameters i.e. color, odor, purchase decision, surface moisture, and meat juice retention was performed. Shelf life was evaluated based on a sensory index method. No significant differences between DLM and DL-HMTBA supplementation could be shown for the investigated meat quality parameters. The supplementation of Met led to heavier fillets, a higher pH-value and a longer sensory shelf life in comparison to the basal group. In contrast, the microbial loads at the end of storage were lower for the basal group, indicating a chemical spoilage process for the basal and a microbial spoilage for the methionine groups. For the drip loss, a significant positive correlation between drip loss and Met supplementation level in DL-HMTBA treated birds could be shown. The development of the sensory index was similar for all treatment groups and Met concentrations. The mean sensory shelf life of the chicken filets varied between 8-9 days. In conclusion, Met supplementation had a significant influence on meat quality parameters. The microbial load, pH and sensory characteristics were positively correlated to the methionine concentration.

Keywords: methionine, methionine hydroxyl analogue, poultry, meat quality, shelf life, sensory quality

S1-0357 D-methionine is as efficient as L-methionine in broiler chickens

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Although early studies reported no significant differences in utilization of DL- Methionine (DLM) and L- Methionine (LM), a few recent studies have concluded that the bio- efficacy (BE) of LM is higher than DLM especially in early ages. To overcome the experimental limitations shown in these studies (high variation and lack of a significant response) and to address the BE at earlier ages, we studied 2120 broilers from 0-28 days. We compared D- Methionine (DM) and LM, as DLM is a 50:50 mixture of both isomers thus, making the detection of true differences easier. Ross 308 male chickens were fed pelleted diet during starter (0-10 days), grower (11-22 days) and finisher (23-28 days) phases. Eight different doses (0.03, 0.06, 0.09, 0.12, 0.15, 0.21, 0.27 and 0.36%) of either DM or LM (6 replicates pens of 20 birds) were added to a basal diet deficient in Met+Cys (0.60, 0.53, and 0.47% in starter, grower, and finisher, respectively). All other amino acids and energy levels were formulated according to AminoChick®2.0 (Evonik, Germany). The non-supplemented group included 10 pens of 20 birds. A dose-response relationship was investigated in a non-linear model simultaneously for both DM and LM. Body weight (BW) and feed intake (FI) were recorded, and body weight gain (BWG) and feed conversion ratio (FCR) calculated. Carcass composition was measured in 24 slaughtered birds per treatment at day 29. No difference between DM and LM was observed with regard to BW, BWG, FI, and FCR (a non-significant relative BE of 102, 102, 99, and 110%, respectively). Slaughter analysis revealed no difference between DM and LM looking into breast muscle yield, thighs, and dressing (a non-significant relative BE of 107, 101, and 113% , respectively). In conclusion, broiler fed with either DM or LM performed equally during their early development. Furthermore, the lack of differences in methionine isomers could be extrapolated to the comparison of LM vs. DLM that only contains 50% DM.

Keywords: D-methionine, L-methionine, broilers, bio-efficacy

S1-0358 DL-methionine outperforms its hydroxy analogue and is as efficient as L-methionine in broiler chickens

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Supplementing methionine sources to broiler diets in order to balance the dietary amino acid according to the broilers demand is a common practice. Various methionine sources are available such as DL- Methionine (DLM), DL-2-Hydroxy-(4-methylthio) butanoic acid (DL- HMTBA) and L- Methionine (LM). In order to effectively use different methionine sources, knowledge about the biological effectiveness is needed. DL- HTMBA has a lower relative bio- efficacy (RBV=65%) in comparison to DLM. Early research showed no difference between LM and DLM. However, newly published data presents a higher RBV of LM vs. DLM in piglets and in broilers. Herein, we aimed to determine the RBV of LM and DL- HMTBA vs. DLM using a dose response experiment. Male Ross 308 chickens (n=2160) were fed pelleted diets during starter (0-10 days), grower (11-22 days) and finisher (23-35 days) phases. Six different doses (0.02, 0.04, 0.08, 0.12, 0.20, and 0.32%; on an equimolar basis) of either DLM or LM or DL- HMTBA (6 replicates of 20 birds; 5 replicates for the last 2 levels) were added to a basal diet. Basal diet was deficient in Met+ Cys (0.60, 0.53, and 0.47% in starter, grower, and finisher, respectively). All diets were isoenergetic and isoproteic with amino acid specification meeting or exceeding AminoChick®2.0 (Evonik Nutrition & Care GmbH) recommendations. Body weight, feed intake, weight gain and feed conversion ratio (FCR) were measured. A dose-response relationship was investigated using a non-linear model simultaneously for all methionine sources. No difference between DM and LM were observed with regard to body weight, body weight gain, feed consumption, and FCR (a non-significant RBV of 103, 103, 100, and 105%, respectively). DLM presented a superiority over DL- HMTBA for the body weight, body weight gain, and FCR parameters (a significant RBV of 82, 82, and 70%, respectively). This study illustrated equality of DLM and LM and provided further evidence of a lower RBV of DL- HMTBA vs. DLM.

Keywords: DL-methionine, L-methionine, methionine hydroxyl analogue (MHA), broilers, bio-efficacy

S1- 0359 Metabolomics revealed equality in utilization of D- methionine and L-methionine and a new insight in sulfur amino acid metabolism in broiler chickens

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Sulfur amino acid (SAA) metabolism has been the subject of extensive research in various species. However, information on the metabolism of SAA in poultry is limited. New technologies have created new opportunities to explore SAA utilization. Herein, we studied the broilers' metabolome, a comprehensive spectrum of metabolites and nutrients, using metabolomics. In a trial comparing D-Methionine (D-Met) and L-Met, blood samples were collected from Ross 308 broilers (n=8) fed for 35 days a Met+Cys deficient diet (0.60, 0.53, and 0.47% in starter, grower, and finisher, respectively; digestible basis) or diets supplemented with submarginal (0.06 and 0.12%) or adequate (0.21%) levels of either D-Met or L-Met. In brief, blood samples were extracted and split into equal parts for analysis on the LC/MS/MS, and Polar LC platforms. One-way ANOVA was used to analyze the data. A total of 780 metabolites were detected (603 known and 177 unknown). Significant metabolites were categorized to specific pathways. Significant pathways ($P < 0.05$) with more than 5 metabolites were compared between groups. At 0.06% submarginal Met+Cys condition, L-Met resulted in lower concentration of long chain fatty acids and monoacylglycerol compared with D-Met. At 0.12% submarginal Met+Cys levels, L-Met down regulated histidine metabolism, caused the oxidation of Met and increased the homocysteine compared with D-Met. At adequate Met+Cys levels, Met oxidation was the major observation comparing L-Met to D-Met fed broilers. A dose dependent reduction in the plasma concentration of histidine, lysine, glycine, serine, threonine, and branched chain amino acids was observed by an increase of either D-Met or L-Met. A specific effect of L-Met supplementation was an increase in 16 different lysolipids, which are known to be result of degradation of phospholipids as a major component of cell membrane. In conclusion, metabolomics revealed a high similarity between D-Met and L-Met metabolism in broiler chickens.

Keywords: metabolomics, D-methionine, L-methionine, chicken

S1-0360 Understanding the relationship of dietary metabolizable energy and ideal protein in modern broilers: a metabolomics insight

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Dietary energy (E) and amino acids (AA) interaction has been studied throughout the years without conclusive findings. We studied birds' metabolome to underpin the reasons. Ross 308 male chickens (n=2160) were divided to 12 treatments (9 replicates each of 20 birds) from 21 to 37 days. Four levels of E (2875, 3000, 3125/adequate, 3250 TMEn kcal/kg) and 3 levels of ideal protein (IP) (85, 100, and 115% of Evonik's AMINOChick®2.0) were used in a factorial design. Per treatment, 5 Birds were slaughtered at day 37 and blood samples were used for analysis on a LC/MS/MS and Polar LC platforms. Data was analyzed using two-way ANOVA. A total of 622 metabolites were detected (523 known and 99 unknown). Significant pathways ($P < 0.05$) with more than 5 metabolites are reported. At lowest E level, increase of IP to 100% had no effect on AA metabolism thus probably majority of AA were used for protein synthesis. Higher dietary IP (115%) increased Lys anabolites and mildly Met oxidation metabolites. At sub-marginal E levels, increase of IP to 100% up-regulated AA metabolism (especially Lys and Met), which means that AA were not only used for protein synthesis but also metabolized. Increasing IP to 115% caused a sharp increase in Lys metabolites and Met metabolism. Moreover, BCAA catabolism was observed. At adequate E levels, increase of IP to 100% was accompanied with a limitation in His and its metabolites (sign of His deficiency). Increase of IP to 115%, caused only a mild increase in Met and Thr related metabolites. Nevertheless, pyrimidine metabolism cytidine and uracil dependent was up and down regulated, respectively. At excess E levels, His was limiting by increase of IP to 100% and Met metabolism was enhanced. Increase of IP to 115% increased Lys anabolites and pyrimidine metabolism (cytidine dependent). In conclusion, metabolomics revealed that AA utilization differs depending on E levels and that histidine needs to be reconsidered especially at adequate and high E levels.

Keywords: metabolomics, energy, digestible lysine, ideal protein, chicken

S1-0361 Fads1 and 2 are promoted to meet instant need for long chain-polyunsaturated fatty acids in goose fatty liver

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Global prevalence of non-alcoholic fatty liver disease (NAFLD) constitutes a threat to human health. Goose is a unique model of NAFLD for discovering therapeutic targets as its liver can develop severe steatosis without overt injury. Fatty acid desaturase (Fads) is a potential therapeutic target as Fads expression and mutations are associated with liver fat. Here, we hypothesized that Fads was promoted to provide a protection for goose fatty liver. To test this, goose Fads1 and Fads2 were sequenced. Fads1/2/6 expression was determined in goose liver and primary hepatocytes by quantitative PCR. Liver fatty acid composition was also analyzed by gas chromatography. Data indicated that hepatic Fads1/2/6 expression was gradually increased with over-feeding time. In contrast, trans-C18:1n9 fatty acid (Fads inhibitor) was reduced. However, enhanced Fads capacity for long chain-polyunsaturated fatty acid (LC-PUFA) synthesis was not sufficient to compensate for the depleted LC-PUFAs in goose fatty liver. Moreover, cell studies showed that Fads1/2/6 expression was regulated by fatty liver-associated factors. Together, these findings suggest Fads1/2 as protective components are promoted to meet instant need for LC-PUFAs in goose fatty liver, and we propose this is required for severe hepatic steatosis without liver injury.

Keywords: cloning, fatty acid desaturase, non-alcoholic fatty liver disease, goose, long chain polyunsaturated fatty acid

S1-0362 The effect of single protease enzyme on metabolic energy and amino acid digestibility in broiler chicks fed diets with soya bean meal and rice bran pure diets

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Standardized ileal digestibility (SID) of amino acid (AA) was quantified in 30-d old Ross 308 male broilers. Experimental design was a 4x2 factorial arrangement of treatments containing 4 doses (0, 0.025, 0.0375, or 0.05% of diet) of an inherently thermostable protease (CIBENZA® DP100, Novus International, Inc.) and 2 ingredients [soybean meal (SBM) or rice bran (RB)]. Birds were randomly assigned to with 6 pens/treatment, 7 birds/pen, and fed diets containing the test ingredient as the sole source of N or a N-free diet for endogenous AA losses. Data were analyzed by ANOVA and means were separated by Duncan's multiple range test. Test diets were fed from 21 to 30 d of age, excreta were collected from 24 to 28 d for AME determination, and ileal digesta was collected for and AA SID on d 30. Protease supplementation linearly increased ($P<0.001$) AME and AMEn in both SBM and RB; the increase was proportionally greater ($P<0.008$) for RB than for SBM with higher protease inclusion. Protease inclusion also linearly increased ($P<0.001$) CP digestibility. Protease linearly increase SID of Met, Trp, Val, Ser, Ala, Tyr, Pro, Cys, NEAA, and all AA in SBM ($P<0.05$) and Met, Arg, Leu, Phe, His, Val, Ser, Glu, Gly, Tyr, Pro, Cys, EAA, NEAA, and all AA in RB ($P<0.04$). Protease tended to linearly increase SID of Lys, Thr, Asp, and EAA in SBM ($P<0.09$) and Trp and Asp in RB ($P<0.08$). Improvements in CP and AA digestibility, AME, and AMEn were negatively correlated with basal AA SID and positively correlated with protease inclusion in diets. Highest digestibility coefficients for CP and AA, as well as, AME and AMEn were quantified for protease included at 0.05% of diet regardless of ingredient tested. In conclusion, protease supplementation increased AME, AMEn, CP, and AA digestibility in both SBM and RB and it is a viable enzyme to increase the metabolizable energy and digestible AA content of diets for broiler chickens.

Keywords: protease, metabolizable energy, amino acid digestibility, broiler

S1-0363 World mycotoxin survey in 2015

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Mycotoxins are a large group of fungal secondary metabolites and are commonly found worldwide in cereal grains and animal feed. The BIOMIN Mycotoxin Survey provides an overview on the incidence of aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM), T-2 toxin (T-2) and ochratoxin A (OTA) in various feed and raw materials. From January to December 2015, a total of 8,271 samples (31,492 analyses) were analyzed from over 75 different countries around the world by HPLC, LC-MS/MS and ELISA. Global: the most common mycotoxins are DON, FUM and ZEN which were detected in 73%, 61% and 56% of all samples with average of positives at 1,090 ppb, 1,089 and 253 ppb, respectively. In 2015, more than 80% of all samples contained at least one mycotoxin. Europe: DON remains the main concern in Europe with 77% of samples contaminated, with average of positive levels at 1,288 ppb. Highest prevalence of T-2 (42%) was observed in Europe with an average of positives at 25 ppb. Asia: Most prevalent mycotoxins in Asia were DON, FUM and ZEN, with prevalence levels at 74%, 67% and 55% respectively, and the average of positive levels at 857 ppb, 1,032 ppb and 368 ppb respectively. North America: Main concern in North America is DON with a prevalence of 67% with an average of positive level at 1,132 ppb. South America: FUM remains the main concern in South America: 70% prevalence and average of positives at 2,235 ppb, also the highest singly occurring FUM (36,489 ppb) observed in a Brazilian corn sample. Middle East: The Middle East shows the highest prevalence of FUM: 84% contamination, followed by OTA (62%), ZEN (54%) and DON (53%).

Keywords: mycotoxins, feed, raw materials, occurrence

S1-0364 Occurrence of mycotoxins in 2015 new harvest corn in China

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A regional mycotoxin survey was conducted with 127 corn samples newly harvested from 11 major production provinces in 2015. Four mycotoxins, namely aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON) and fumonisins (FUM) were analyzed for each sample by HPLC. In northeast region (Heilongjiang, Jilin and Liaoning), occurrence of DON and ZEN has been largely increased compared with last year. Above 90% of all tested samples from these provinces have been contaminated with DON, with an average of positive level in between 320-481 ppb. In central west provinces like Shanxi and Shaanxi, the most prevalent mycotoxins are FUM, DON and ZEN, with positive percentage at 100%, 63% and 50% respectively, and the average of positive levels at 1,804 ppb, 914 ppb and 179 ppb respectively. In central east provinces like Hebei, Shandong and Henan, all Fusarium toxins are highly prevalent, with occurrence levels at 98%, 87%, 77% and average of positive levels at 3,451 ppb, 441 ppb and 184 ppb for FUM, DON and ZEN respectively. While the risk levels of ZEN and DON were slightly below year 2014, the contamination level of FUM has been drastically increased. In addition, attention should also be paid to Afla from south of Henan and Shandong provinces. Highest Afla and FUM have been found in central south provinces including Hubei, Jiangsu and Anhui. The positive percentage were 61% and 100% with average of positive levels at 108 ppb and 4,069 ppb respectively. DON and ZEN are also at threshold levels with a similar profile as central east region.

Keywords: mycotoxin, occurrence, China, corn

S1-0365 Effect of body weight at 15 weeks age on egg laying performance of CAU No.3 Layer at 21-27 weeks

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The experiment was conducted to explore appropriate weight of CAU No.3 Layer at 15 weeks age and guide diet ration. According to body weight at 15 weeks age, 1080 CAU No.3 Layer were divided into six groups, respectively less than 700g, 700- 799g, 800- 899g , 900- 999g, 1000- 1099g, more than 1100g, each group had four replicates. The result suggested that age at first egg was delayed, weight at first egg was smaller, hen-day egg production and hen-day laying rate from 21 to 27 weeks age was obviously lower than other groups when body weight was less than 800g($P<0.05$). The egg production was 26.19, the laying rate was 53.44% at the weight of less than 700g. While the egg production could reach 36, the laying rate could reach 74% at the weight of more than 1000g. The correlation between body weight with average egg weight and daily egg mass was positive. When the body weight was less than 700g, the average egg weight was only 39.37g; For every 100g increase in body weight , egg weight increased by more than 1g. Average egg weight and daily egg mass was obviously higher than other groups with body weight at more than 1000g($P<0.05$). Feeding CAU No.3 Layer, it should be limited more than 800g; When body weight was more than 1000g, age at first egg and weight at first egg was suitable, egg laying rate increased fast, which would be benefit to enhance egg production and egg laying rate, increase egg mass.

Keywords: CAU No.3 Layer, body weight at 15 weeks age, egg laying performance

S1-0366 The effects of different inorganic phosphorus sources in the diet on production performances of broilers

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The objective of this study was to compare the influence of phosphorus from different dietary supplements of mineral P sources, on production performance of broilers. The effects of a supplementation of P from monobasic calcium phosphate (MCP; $\text{Ca}(\text{H}_2\text{PO}_4)_2$) to low-P basal diets were studied in growing broilers. 300 broilers (Cobb 500), both sex, from the same hatch are used in the trial. During the trial, chickens are divided in 3 groups, with 3 different experimental diets. The low-P basal diet was formulated without addition of monobasic calcium phosphate, and the other 2 diets were formulated with addition of 2 different MCP (one was provided by Eliksir Group D.O.O., Sabac, Serbia, and the other one was originating from Russia). During the experiment, that lasted 42 days through three phases (1-21, 21-35 and 35-42 days), performances, health status and mortality were monitored. Broilers fed with addition of MCP improved average body mass, average daily gain, feed intake and feed intake to body weight gain ratio. ($p<0.05$) The results of the experiment point to the response of broilers on dietary P in the sense of improving production results - increase in body weight, and body gain and decrease in feed consumption and feed conversion, which indicates the significance of the availability of P from mineral nutrients. Due to requirement of growing animals for P, which they need to maintain commonly known metabolic functions and skeletal development, but also due to its low availability from plant ingredients (which are the basis for compound chicken feed) inorganic phosphates are, widely used to increase the concentration of available P in the diet. Therefore, it is of crucial importance to determine the effects of P from these types of supplements on growth and production performances of broilers.

Keywords: broiler, phosphorus, MCP, production results

S1-0367 Prokaryotic expression, purification and bioactivity identification of recombinant chicken Visfatin protein

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Visfatin is a novel adipocytokine which was firstly identified in 2005. In recent years, some research showed that human recombination Visfatin could enhance the feed intake of chicken. By far, the avian Visfatin has not been cloned and expressed in vitro, however. For obtaining chicken Visfatin protein to use in molecular studies related to poultry, we constructed a prokaryotic expression plasmid pET30a-visfatin, optimized expression conditions in E.coli, purified the surviving recombinant protein and identified its bioactivity. In this study, the CDS sequence of Visfatin was amplified by RT-PCR and cloned into prokaryotic expression vector pET-30a which was transformed into E.coli BL21 (DE3) for expression. After optimizing induction conditions, including PH, time, temperature and concentration of IPTG, the recombinant protein was purified using Ni-affinity chromatography with 50mM and 150mM imidazole. The product of expression and purification was identified by SDS-PAGE and Western Blot, and the bioactivity was detected in 3T3-L1. As expected, we constructed the pET-30a-Visfatin expression plasmid successfully. The optimum condition of induction was 0.4mM IPTG, 30°C and PH8 for 12h. The molecular weight of the purified recombinant protein on SDS gels matched the predicted protein (60kDa). Visfatin was confirmed by Western Blot analysis via specific binding to the 6*His tag. Additionally, the results of oil red O stain showed that there were more lipid droplets formation in 3T3-L1 induced with Visfatin than the control. During the differentiation of 3T3-L1, Visfatin protein increased the expression of marker genes significantly ($P < 0.05$), such as PPAR γ , aP2, FAS and C/EBP α . These results indicated that the chicken Visfatin protein has biological function. In conclusion, we established a standardized prokaryotic expression program of the active chicken recombinant Visfatin protein, which laid a foundation for further research in the field of poultry.

Keywords: chicken recombinant Visfatin, prokaryotic expression, condition optimization, bioactivity identification

S1-0368 Evaluation of yeast sludge cell wall and commercial clay based toxin binders against aflatoxin B1 on growth and serum parameters of broilers

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A comparative study was conducted to evaluate yeast sludge cell wall (YSCW) and commercial toxin binders against different levels of aflatoxins B1 (AFB1) in broilers. A total of 390 1-d-old chicks were divided into 13 treatments (three replicates / treatment ten birds in each). Negative control (NC) contained no AFB1 and toxin binder in the feed. Positive control (PC) was offered with different AFB1 levels (100, 200 and 300 $\mu\text{g/kg}$) in feed. The remaining treatments included YSCW, bentonites and, combination of bentonites and glucomannan (Bent + Gluc) at different AFB1 levels in factorial arrangement from 8th to 28th days of age. Among positive control, 200 and 300 $\mu\text{g/kg}$ AFB1 levels showed 37.42% and 36.38% decrease in weight gain causing 1.41 and 1.35 times increase in feed conversion ratio (FCR) compared to Negative control. YSCW and Bent + Gluc showed better weight gain (87.9, 83.45 and 75.48% for YSCW and 83.37, 82.83 and 78.35 for Bent + Gluc vs 66.26, 62.6 and 63.6% for PC as compared to NC) at 100, 200 and 300 μg AFB1/kg respectively. The dietary treatments, aflatoxin (AFs) levels and toxin binders showed non-significant effect for feed intake, serum ALT and significant on albumin and uric acid. The AFs levels, toxin binders and toxin binders \times AFs showed non-significant effect for liver magnesium and copper whereas dietary treatments and AFs showed significant effects on liver zinc. It was concluded that AFB1 affect the growth at different levels significantly. Like other toxin binders, YSCW was found to nullify the deleterious effects of AFB1 and showed non-significant effect among themselves at different AFB1 levels.

Keywords: aflatoxins, broiler, feed intake, mannan oligosaccharide, sonication, yeast sludge cell wall

S1-0370 Effect of BM1259 contrast yucca and bacillus subtilis on production performance and fecal ammonia nitrogen concentration of laying hens

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This research was carried out to investigate the effect of *Bacillus Magaterium* 1259 (BM1259) contrast with *Yucca* and *Bacillus Subtillis* on performance and ammonia nitrogen content in the excrement of laying hens. A total of three hundred and eighty four 300-day-old healthy Hy-line Brown laying hens with similar rate of laying were selected and randomly assigned into four groups including control group and three treatment groups. Each group had six replicates (sixteen hens per replicate). The control group were fed with basal diet and the treatment groups were fed with BM1259, *Yucca* and *Bacillus Subtillis* (all 100mg/kg) based on the basal diet individually. The results showed that 1) supplementing with BM1259 increased average egg weight ($P < 0.019$), number of egg laying ($P < 0.01$), laying rate ($P < 0.01$), and decreased feed to egg ratio ($P < 0.01$), which was similar to *Bacillus Subtillis*, better than *Yucca*. 2) In addition, concentration of $\text{NH}_3\text{-N}$, uric acid, urea nitrogen, and uricase activity were reduced ($P < 0.01$) with BM1259, which was similar to *Yucca*, better than *Bacillus Subtillis*. 3) BM1259 has more profit than the other treatments in this study. Results suggest that supplemented with BM1259 can improve performance, increase profit and better remove ammonia nitrogen and odor in the excrement of laying hens.

Keywords: *Bacillus Magaterium*, *Yucca*, *Bacillus Subtillis*, laying hens, ammonia nitrogen content

S1-0371 Contamination of mycotoxins in feed samples collected from China 2015

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Mycotoxins are highly toxic secondary metabolites produced by fungi, and occur in all kind of feed commodities. These fungal metabolites pose a risk to human and animal health. The focus of this study is to compare the extent of mycotoxin contamination in finished feed and cereals intended for animal feed. As part of the 2015 Biomin mycotoxin survey, 1399 feed and raw material samples from China were tested using high performance liquid chromatography. Major mycotoxins investigated were deoxynivalenol (DON), zearalenon (ZEN), fumonisins (FUM), aflatoxins (Afla) and ochratoxin A (OTA). In total, 94% of samples tested were positive for the major mycotoxins and out of those 62% were co-contaminated with more than one type of mycotoxin. DON was the most frequent mycotoxin present with a prevalence of 90% and was followed by ZEN with 61% of all samples, at an average contamination level of 1023, 509 ppb, respectively. FUM was found in 58% of total samples at an average contamination level of 1881 ppb. Afla (153 ppb) and OTA (18 ppb) were detected in 12% and 18% of all samples, respectively. 73% of corn samples and 63% of finished feed samples were co-contaminated with more than one type of mycotoxin. However, wheat and rice samples showed a relatively low co-occurrence of more than one mycotoxin, with 18% and 25% respectively. The survey results indicate a notable high mycotoxin contamination in samples collected in China. DON, ZEN and FUM were the most frequently occurring mycotoxins, which pose a relatively high threat to animal health. Occurrence of more than one mycotoxin was observed in more than half of the samples. Due to potential additive and synergistic effects, special attention should be paid to co-occurrence of mycotoxins in animal feed (Grenier and Oswald, 2011).

Keywords: mycotoxins, survey, feed, raw materials, China

S1- 0372 Efficacy of L- methionine compared to DL-methionine in broiler chicken

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The current study was designed with the aim to compare the relative bio- efficacy (BE) of the newly developed L-methionine (Met) compared to the conventional DL-Met based on growth performance and carcass composition in broiler chicken. A total of 1456 male Arbor Acres broilers were randomly allotted into 13 dietary treatments with 1 methionine- deficient basal diet without supplementation of Met, and 12 Met-supplemented diet with 6 graded levels of DL-Met or DL-Met on the top of basal diet. The Met content in basal diets for starter and grower were 60% and 65% of the overall Met requirement. And the addition levels of Met kept the total Met content amount to 75, 80, 85, 90, 95 and 100% of the Met requirement for AA broiler. Each treatment contained 8 replicates with 14 birds each. The experiment lasted for 42 days (1~21 d, starter phase; 22- 42 d, grower phase). The results showed that L-Met had much higher bio-efficacy over DL- Met during starter phase (141.5% for ADG, 189.8% for FCR) than during the whole 42 d (123.6% for ADG, and 140.0% for FCR). The average BE of ADG and FCR for the 42 d was 131.8%. Both DL-Met and L-Met decreased blood protein carbonyl content, and L- Met groups had further reduced blood carbonyl content than corresponding DL-Met groups. Overall, under the current experimental condition, BE of L- Met over DL- Met were as follows: 166% (starter phase) and 130 % (whole phase) based on growth data, and 116% and 120% based on breast muscle yield. Met supplementation decreased blood uric acid, uric nitrogen and protein carbonyl content, irrespective of the sources (DL or L isomers).

Keywords: L- methionine, DL- methionine, bio-efficacy, broiler chick, growth performance, breast muscle yield

S1- 0373 Effects of dietary β - alanine on growth performance, meat quality and muscle- derived active peptide in broiler chicken

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Effects of dietary β -alanine on growth, meat quality and muscle-derived active peptide in broiler chicken were investigated in the current study. The study consisted of 2 experiments. Experiment 1 examined the suitable dietary dosage of β -alanine with regard to growth and slaughter performance. Experiment 2 investigated the suitable dosage of β -alanine on growth performance, meat quality and muscle-derived peptide. In experiment 1, 240 one-day-old female Arbor Acres (AA) broilers were randomly allotted into 4 groups with 6 replicates for 6 weeks. Chicks were fed a basal diet supplemented with 0, 200, 400 or 600 mg/kg β -alanine. Results showed that β -alanine at 600 mg/kg level significantly increased the body weight at 42 d of age, enhanced average daily gain during the grower period (22~42 d) and the whole period (1~42 d) ($P < 0.05$). Dietary 400 or 600 mg/kg β -alanine addition significantly increased feed efficiency during the 42 d and promoted growth of breast muscle especially at 600mg/kg level ($P < 0.05$). In experiment 2, a total of 180 one-day-old female AA broilers were randomly divided into 3 groups with 6 replicates and fed diet supplemented with 0, 500 or 1000 mg β -alanine in per kg feed. The results showed that diet supplemented with 500 or 1000mg/kg β -alanine significantly improved ADG during growth period and whole period ($P < 0.05$), and significantly improved feed efficiency during starter, grower and whole period ($P < 0.05$). Chicks fed 500 mg/kg β -alanine diet had superior growth performance to other groups. Compared to control group, supplementation of 500 mg/kg β -alanine significantly increased concentration of carnosine in breast muscle (from 2680.5 to 2998.4 nmol/g tissue), and decreased the shear force and drip loss in breast tissue ($P < 0.05$). In conclusion, dietary supplementation of β -alanine could promote the growth performance, increase the content of functional carnosine of breast muscle and improve meat quality in female broiler chickens.

Keywords: Beta- alanine, growth performance, muscle-derived active peptide, meat quality, broiler

S1-0374 The effect of combining microbial phytase, protease and carbohydrase on performance of broiler chicks fed diets containing reduced levels of available phosphorus, amino acids and energy

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Two studies were conducted to evaluate the effects of supplementing a microbial 6-phytase (CIBENZA® PHYTAVERSE® G10) in combination with a protease (CIBENZA® DP100) and a mannanase (CIBENZA® DE200) in broilers. Study-1 had Ross-308 male chicks, 6 treatments (Trts), 12 pens/Trt and 21 chicks/pen. Trts include reduced levels of available P (aP), amino acids (AA)/CP and energy from the +control (T6). Test diets included: T1 (-control); T1+phytase @500U/kg diet, T2; T1+protease @0.05% of diet, T3; T1+phytase @500U/kg diet + protease @0.05% of diet, T4; T3 + 0.15% aP from di-calcium phosphate, T5; industry levels of aP, AA/CP, and energy, T6 (+control). Study-2 had Cobb-500 straight-run chicks, 6 Trts under 2x3 factorial with 6 pens/Trt and 45 chicks/pen. The 2 levels of mannanase are 0 and 800u/kg diet with no matrix credit and 3 diets types are; 1) +Ctrls, 2) -Ctrls with less AA & ME than +Ctrl, and 3) -Ctrl plus 500g/MT of protease. Phytase was added across diets @1000u/kg diet with matrix values. Study-1 data was subjected to 1-way ANOVA and study-2 to 2-way ANOVA. For study-1 at d28 the gain, FCR and performance index (PI) for T1 was lower compared to T2, T4, T5, and T6 ($P < 0.05$) but similar to T3. Phytase addition to T1 improved ($P < 0.05$) gain, FCR and PI but the response was lower than T4 ($P < 0.05$). When both enzymes were combined (T4) gain, FCR and PI were higher ($P < 0.05$) than addition of either of individual enzymes (T2 or T3). Further, T4 responses were not different from +ve control (T6). At d42 only PI response was similar to d28 response. In study-2, only main effect of protease was significant ($P < 0.01$) for both gain and FCR. The -Ctrl was not different from the +Ctrl but adding protease to -Ctrl improved gain and FCR that are different from -Ctrl ($P < 0.05$) and comparable to +Ctrl ($P > 0.05$). In summary, supplementing both phytase and protease with their full nutrient values led to improved performance beyond that achieved with either enzyme supplemented separately.

Keywords: phytase, protease, mannanase, broiler

S1-0375 Effect of dietary grape proanthocyanidins (GPC) and its active ingredients on growth performance and intestinal immunity in broiler chicks exposed to coccidial infection

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Current study was designed to investigate the effects of mixture or active ingredients of grape proanthocyanidins (GPC) on growth performance and intestinal immunity of broilers exposed to coccidial infection. A total of 540 1-day-old Arbor Acres male broiler were allotted into 6 treatments by a factorial arrangement with 3 diets and 2 types of challenge. Each treatment contained 6 replicates with 15 birds. Diets were supplemented with 15 mg GPC or 1 mg active ingredients of GPC. Birds were inoculated with 1 mL 5×10^4 Eimeria tenella or 1 mL of saline at 14 d. Results showed that coccidial infection decreased ADG and feed efficiency especially in control group, and birds fed GPC mixture or GPC active ingredients attenuated the growth inhibition by coccidia. GPC groups decreased intestinal lesion score, bloody diarrhea and oocyst excretion count. Coccidia significantly elevated the number of $\gamma\delta$ T cell in blood and intestine, while GPC or its active ingredients especially the latter further increased $\gamma\delta$ T cell. In the absence of coccidia, active ingredients of GPC partially increased intestinal $\gamma\delta$ T cell. Upon infection, intestinal $\gamma\delta$ T cell produced more IL-2, IL-4 and IL-12 in GPC mixture or its active ingredients group than the control; IL-12 and IFN- γ mRNA expression in $\gamma\delta$ T cell from chicks fed active ingredients of GPC were higher than those fed GPC or control diet. Coccidia stimulated the ileum epithelial cell and elevated the transforming growth factor- β , epidermal growth factor and secretory immunoglobulin A (sIgA) content in ileum. Chick fed mixture or active ingredients of GPC further stimulated the intestinal immunity, and sIgA elevation was higher in active ingredients group than mixture group. Taken together, GPC mixture or its active ingredients ameliorated the intestinal immunity and attenuated the growth inhibition by coccidial infection in broilers. The active ingredients of GPC had superior anti-coccidial infection efficacy over the GPC mixture.

Keywords: grape proanthocyanidins, active ingredients, growth performance, intestinal immunity, broiler chicken

S1-0376 Effect of two doses of exogenous xylanase, amylase and protease combination on ileal nutrient digestibility of turkeys fed corn or wheat based diets

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Two simultaneous trials were conducted to evaluate the effect of two doses of exogenous xylanase (X), amylase (A) and protease (P) combination on ileal nutrient digestibility of turkeys fed either corn or wheat based diets. 320, 28d old male Nicholas turkeys were allocated to 64 cages. Ileal digesta was collected on d42 for the determination of nutrient digestibility. For each grain type, a nutritionally adequate, positive control (PC) diet was formulated. The negative control (NC) diet was formulated to be lower in metabolizable energy (~86 or 108 kcal/kg diet for corn and wheat, respectively) and amino acids compared to PC. The NC diet was supplemented with a combination of X, A, and P at either 50 or 100 g/MT (100g/MT provided 2000 U of X, 200 U of A, and 4,000 U of P/kg diet). All diets contained phytase (500 FTU/kg) which contributed 0.15% AvP and 0.13% Ca. Data were subjected to one-way ANOVA and means were separated by Student's test. In both grain types, the combination of X, A and P at 100g/MT improved ($P < 0.05$) protein digestibility (11.7 and 10.6%, for corn and wheat, respectively) and mean amino acid digestibility (7.9 and 9.3%, for corn and wheat, respectively) compared to NC. Enzyme supplementation at 50 g/MT had no effect ($P > 0.05$) on protein and mean amino acid digestibility compared to NC and 100g/MT inclusion level. Dry matter, gross energy and minerals (Mg, K and S) digestibility were improved ($P < 0.05$) by enzyme supplementation at both doses in the wheat based diets only. In conclusion, enzyme combination of X, A, and P improved ileal protein and mean amino acid digestibility in a dose response manner in turkeys fed either corn or wheat based diets. Enzyme response on dry matter and gross energy digestibility was observed in the wheat based diet only.

Keywords: turkeys, ileal digestibility, xylanase, amylase, protease

S1-0377 Effect of encapsulated protease and amylase enzymes on the performance of broilers

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Enzymes are thermo-labile hence there may be loss of enzyme activity during pelletization of feed. Encapsulation or coating of enzymes is important for stability of feed enzymes. Protease and Amylase enzymes were encapsulated/coated with sodium alginate and included in corn-soya pelleted diet at 6000 IU/kg and 2000 IU/kg respectively. Ten treatments including one control (22% CP, 2900 kcal ME starter, 19% CP, 3100 kcal ME finisher) diet and three basal diets i.e. basal low CP (2% less protein, ME remains same as control), basal low ME (115 Kcal less ME, CP remains same as control) and basal low CP and low ME (2% less CP, 115 kcal less ME over control) were formulated and six more diets formulated by supplementing each of the two enzymes separately to three basal diets. 500 day old broiler chicks were randomly allocated into ten treatment each with ten replicates with five birds in each replicate. Addition of coated or uncoated enzymes to their respective basal diets had shown no significant influence on weight gain, feed intake, livability and carcass parameters of broilers chicks during starter, finisher, and over all period when compared to Control group. But Supplementation of uncoated or coated enzyme yielded significantly better FCR over the control diet and their respective basal diets during overall period with improved digestibility of crude protein and energy compared to their basal diets. Enzyme supplementation has resulted an improved performance in broilers through better FCR, digestibility. Reduction in abdominal fat was evident in amylase supplementation to low ME diets.

Keywords: broilers, coated enzymes, protease, amylase, growth performance

S1- 0378 Effect of feeding various probiotics on performance, intestinal micro flora and fecal gas emission of broiler chicks

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This study was conducted to investigate the feeding influence of various probiotics on performance, intestinal microflora, blood properties and fecal gas emission of broiler chicks. Three hundred twenty one day old Ross×Ross broiler chicks were placed into five treatments with four replicates in the floor pen for five weeks. Treatments were divided into control, antibiotic Avilamycin, P (probiotics), PS (Probiotics with sulfone) and PG (probiotics with Ginseng) were inoculated 0.1% level in the diet. Weight gain, feed intake were measured weekly. Blood, spleen and feces were collected at the end of experiment. Results showed that weight gain, feed intake and conversion were not statistically different among the treatments. Blood properties were not altered by the treatment groups. The proliferation of spleen was significantly increased by the P treatment compared to that of other treatments ($P<0.05$). The number of salmonella spp. and E. coli of ileal intestine was significantly higher in control group than those of other treatments ($P<0.05$). Lactobacillus spp showed significantly higher number in P treatment than other dietary treatments ($P<0.05$). Fecal ammonia and CO₂ emission was significantly decreased in PG treatment ($P<0.05$) than that of control group. Thus, P and PG would be valuable feed additives to improve the growth performance, lactobacillus proliferation and immunity of broiler chicks.

Keywords: probiotics, performance, intestinal microorganisms, immunity, broiler chicks

S1- 0379 Effect of feeding various probiotics on performance, blood properties, egg quality and yolk fatty acid composition of laying hens

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The present experiment was conducted to evaluate the effects of different kinds of probiotics on the performance, blood properties, egg quality and yolk fatty acid composition of laying hens. A total of 360 Lohmann light laying hens were randomly divided into 4 groups, and 5 replicates of 18 birds in each replicate pen. Birds had ad libitum access to feed (contain 2740 kcal/kg ME and 16.2% CP) and water throughout the study (29-50 weeks). Treatment included 1) control (basal diet without probiotics), 2) inoculation of 0.1% P (probiotics) with basal diet (B), 3) inoculation of 0.1% PG (Probiotics with Ginseng) and 4) inoculation of 0.1% PS (Probiotics with Sulfone) in the diet respectively. Egg production, egg weight and feed intake in each treatment were recorded daily and egg quality were measured every four weeks interval. Results indicated that 0.1% PG supplemented with basal diet had numerically increased egg production. Feed intake was significantly reduced by the probiotics feeding. Egg weight, egg mass and feed conversion ratio were not influenced by the supplementation of probiotics into the diet. In egg quality, egg shell color, albumen height, Haugh unit, yolk color and egg shell strength were not altered by the probiotic treatments. But, serum total cholesterol (72.2 mg/dl) and triglycerides (764.51 mg/dl) content was reduced significantly by addition of PG into the diet compared to control (total cholesterol 117.6 mg/dl and triglycerides (1276.80 mg/dl). On the other hand, saturated and unsaturated fatty acid contents were not influenced by the feeding of probiotics in broiler diet. In conclusion, the data indicated that feeding dietary supplementation of 0.1 % PG probiotics did decrease serum cholesterol without affecting performance and egg quality of laying hens.

Keywords: probiotics, performance, egg quality, blood properties, laying hens

S1- 0380 Modelling lysine requirements in broiler breeder hens based on potential for nitrogen retention and efficiency of dietary lysine utilization

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The current feeding programs used for broiler breeder hens need more information how to make use of the genetic potential more efficiently. The study aimed to apply modelling of hen's amino acid (AA) requirements and estimated model parameters for maximum nitrogen retention (NRmaxT), nitrogen maintenance requirement (NMR) and lysine (Lys) efficiency (bc-1) to conclude Lys requirement data of broiler breeder hens. Nitrogen balance trials were performed in two periods (I: 31-35 wks and II: 46-50 wks). Seven graded dietary protein contents were examined (eight individual hens as replicates per diet). The protein content of final diets ranged between 58.8 and 311.9 g/kg as fed. Lys was the limiting AA in the dietary protein ($c = 3.91 \text{ g Lys/100g CP}$). For each period, nitrogen intake (NI), nitrogen excretion (NEX), nitrogen in egg mass (NEM), nitrogen deposition (ND, $\text{ND} = \text{NI} - \text{NEX}$) and nitrogen retention (NR, $\text{NR} = \text{ND} + \text{NEM} + \text{NMR}$) were measured in a 25d balance trial. The actual NMR was derived by the exponential relationship between NEX and NI. The NRmaxT and b (slope related to dietary protein quality) were estimated by the exponential fit between NR and NI. Based on the likelihood ratio test for the model parameters, the obtained values were 255 mgN/BWkg^{0.67} for NMR, 0.000117 for b and 1684 mgN/BWkg^{0.67} (period I) and 1484 mgN/BWkg^{0.67} (period II) for NRmaxT. The model derived daily Lys intakes for 80% of NRmaxT of breeder hens in the periods I and II were 915 and 876 mg/BWkg^{0.67}, respectively. The current study concludes that the observed optimal Lys supply is in range with literature data, but the recommendations can be adapted according to feed intake, aimed protein deposition and dietary AA efficiency by modelling.

Keywords: dilution technique, egg mass, exponential, lysine, maintenance

S1-0381 Evaluation of dietary inclusion levels of Biostrong® 510 as replacement for antibiotic growth promoters in broiler chickens production under field conditions

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A feeding trial was conducted to evaluate the optimum inclusion levels of Biostrong® 510 (B510) in the diets of broiler chickens as replacement for antibiotic growth promoters (AGPs). Three hundred and ninety six day old broiler chicks were randomly assigned to six experimental diets with three replicates (of 22 chicks each) per treatment. T1, T2, T3 and T4 diets were supplemented with 0g, 15g, 17.5g, and 20g B510 per 100 Kg diet respectively while T5 had feed grade Oxytetracycline and T6 had neither B510 nor Oxytetracycline but birds were administered water grade antibiotic, Neocylil® plus. Growth parameters taken include initial weight, final weight, feed consumption, FCR, feed cost/Kg and feed cost/Kg gain. At 28 days of age, blood samples were collected over EDTA to evaluate blood quality indices and samples without anticoagulant for liver function indices. The quality of the tibia bone was evaluated. All data generated were subjected to analysis of variance and difference in means was compared using Dunnett's test. Final weight, weight gain, FCR and feed cost/Kg gain were not significantly ($P > 0.05$) different at 28 days. Daily feed consumption was significantly ($P < 0.05$) higher for T1, and T6 than T2, T3 T4 and T5. PCV, Hb, and RBC were significantly ($P < 0.05$) higher for T1, T2, T3, T4 and T6 above T5 while Tp and WBC was not significantly ($P > 0.05$) different. For the liver function test, Glucose, Albumin, AST, ALT and blood urea nitrogen were not significantly ($P < 0.05$) different but ALP was significantly higher for T1 and T6 over the other treatments and least for T4. Bone density, dry matter and percent ash were not significantly different for all the treatments. Biostrong® 510 has potential to improve blood quality, liver performance and bone quality in terms of bone dry matter and bone density.

Keywords: Biostrong®510, performance, blood quality, liver function, tibia quality

S1-0382 The effect of multi-strain probiotic SYNLAB® II on productivity performance of commercial poultry

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The objective of this study was to investigate the effect of feeding probiotic on the performance of layer hens and commercial broiler duck. In the study of layer hens, total of 224 Hy-Line W36 hens at 20 weeks of age were randomly distributed into two groups fed basal diet or basal diet plus multi-strain probiotic SYNLAB® II at 106 CFU/g of the feed. The egg production performance during early (20-34 wks), mid (43-52 wks) and late (70-80 wks) laying periods were determined separately. During early laying period, the supplementation of SYNLAB® II significantly improved feed conversion ratio (FCR) ($P < 0.05$) compared to control. Moreover, the level of antibody titer against ND was significantly stable in SYNLAB® II group ($P < 0.05$). The egg production was significantly enhanced in SYNLAB® II group during mid and late laying periods ($P < 0.05$). At late laying period, the average egg productions were 86.7% and 83.7% in SYNLAB® II and control group, respectively. In broiler duck experiment, the potential of SYNLAB® II as a substitute for antibiotic growth promoters (AGPs) in the production of boiler ducks was evaluated at thirteen commercial duck farms in China, at an average of 10,000 to 20,000 Cherry Valley ducks per farm per group. SYNLAB® II was supplemented in drinking water at a dosage of 100g/5000 birds per day and the production parameters, including body weight, feed intake, FCR, mortality and drug consumption during the whole production period of 36 days were compared with AGPs group. The result showed that supplementation of SYNLAB® II had better FCR and mortality rate than AGPs at 46% and 53.4% of duck farms, respectively. The use of medication was also reduced in SYNLAB® II group at all farms. From all the 13 duck farms, the production cost reduced 474 US dollars per 10,000 birds when SYNLAB® II was used in replacement of AGPs. From the results, SYNLAB® II could improve the immunity and productivity of poultry and as an alternative of AGPs.

Keywords: probiotic, poultry, layer hen, duck, antibiotic growth promoters

S1-0383 Responses of Japanese quails to methionine intake

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The objective of this study was to determine the optimal methionine intake for Japanese quails, using the association of polynomial quadratic with the plateau of the broken-line model. Three hundred ninety two Japanese quails with 16 weeks of age were distributed in a completely randomized design with seven treatments (methionine levels), seven replicates and seven quails per experimental unit. A diet with high protein level (summit) and a nitrogen-free diet were formulated. Both diets had the same level of minerals, vitamins and energy. The methionine content of the summit diet was 13 g/kg, considering 1.2 times the methionine requirement recommended by the Brazilian Tables and 1.4 times the requirements of the remaining amino acids to create a relative deficiency of 20% of methionine. The intermediary methionine levels (2.70; 4.32; 5.40; 6.80; 8.10; 9.50 and 10.79g/kg) were obtained by successive dilutions of the summit diet with the nitrogen-free diet. The eighth treatment was added (1.56g of DL-Methionine, in 0.270 diet) to confirm if methionine was the first limiting amino acid. The trial lasted three weeks of adaptation and four weeks of data collection. The additional response observed in the eight treatment confirmed that methionine was the first limiting amino acid in the diets. The variables measured in this study were feed conversion (g/g), egg production (%) and egg mass (g). The first intersection of the polynomial quadratic curve with the plateau of the broken-line model was calculated in order to determine the optimum methionine intake that provide the maximum feed conversion, egg production and egg mass. Thus, it was estimated the methionine intake of 148; 180; 204 mg/quail/day for feed conversion, egg production and egg mass, respectively. In conclusion, we recommend using 204 mg/quail/day or 0.960% of digestible methionine for Japanese quails to maximize egg mass due to the market's interests.

Keywords: dilution technique, amino acid requirements, models

S1-0384 Use of herbal non antibiotic growth promoter (NAGP) to effectively replace antibiotic growth promoter (AGP) in broiler production

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Latest trend in broiler production is to reduce the use of feed antibiotics to prevent antibiotic resistance. Complete withdrawal may lead to exposing birds to GI tract infections, hence efforts are on to identify phytobiotic products as standardized plant extracts as safe replacement of antibiotics. The trial was conducted to find efficacy of a product developed by Varsha Multitech, Bangalore, India, by supplementation of NAGP (Grostim) compared to negative Control diet without AGP and positive control diet with AGP (Avilamycin). Corn soya based diets were accordingly prepared. The trial was conducted on Cobb 400 broiler chicks randomly assigned to 3 treatment groups in 6 replication of 60 chicks each on August 15, 2015. Three groups were raised on similar management practices in an open sided shed on deep litter. Feed allocation and mortality were recorded daily and body weights were taken on 10% random picked birds at weekly interval. One random bird from each pen was sacrificed every week from 2 to 5 weeks to study intestinal morphology and weights of immune system organs like liver, gizzard, spleen and bursa were recorded and standardized as ratio of live weight of the bird concerned. The data were subjected to statistical analyses as suggested by Snedecor and Cochran (1994). The AGP and NAGP regimes improved all performance parameters compared to negative control regime. EPEF readings in three regimes were 303.52, 340.83 and 275.8 respectively thus showing 10% and 23.58% improvement in AGP and NAGP groups respectively. There was improvement in immune system development. Organ weights were higher in these two groups and spleen / bursa ratio was narrower compared to negative control regime. Both regimes also resulted in improved gut tissue health as evident from lower Villi height/Crypt depth ratio. Overall NAGP regime was as effective as AGP and both gave superior results compared to negative control. The trial proved NAGP as an effective replacement for AGP.

Keywords: AGP, NAGP, growth promoter

S1- 0385 Broiler performance with or without choline chloride or with lipocare, a choline chloride replacer

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Broiler chicks were subjected to 3 types of diets for comparative performance study over 35 days. 150 day old chicks of Cobb 400 variety were randomly assigned to 3 pens of 50 each. These were housed at 1sq ft floor space each in open sided house on deep litter at Nandana Research & Training Institute, Karnataka during the period Oct - Nov 2015. First group was Control feed with Choline Chloride (CC 60%) at the inclusion rate of 1 kg /ton in starter feed and 1.5 kg/ton finisher feed, second group was negative control without any CC 60% and third group received a feed fortified with Lipocare (Varsha Multitech) 1kg/ton starter feed and 1.5 kg finisher feed. The trial was conducted to find out usefulness of CC in broiler production and efficacy of Lipocare, a replacer of CC. Mortality and feed consumption records were kept on daily basis, weekly body weights were taken on random 20% chicks. 5 chicks from each group were slaughtered on day 35 and dressing yield with skin, giblets and neck were estimated. Abdominal fat content was expressed as % of live weight. Treatment effects on 5th week live weight and FCR were statistically analyzed using Completely Randomized Design (CRD). Av. Live weight for CC group was 1923.33g, 1877.50g for No CC group and 2051.67g for Lipocare group. 5th week cumulative FCR figures were 1.62, 1.65 and 1.53 for three groups. Differences between CC and no CC group were statistically not significant. Lipocare was significantly superior to both groups. EPEF was calculated for each group at day 35. CC group had EPEF 332, No CC group 320 and Lipocare group 372. Dressing yields were 76%, 78% and 82%. Abdominal fat expressed as % live weight were 0.93, 0.98 and 0.54 for 3 groups. The trial confirmed the usefulness of CC in broiler production. It was concluded that Lipocare can not only effectively replace CC in diet but also provide several benefits to overall production performance of broilers.

Keywords: choline chloride, choline replacer

S1-0387 Effect of the acidified drinking water on growth performance of Cherry Valley ducks from 1 to 35 d of age

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This study included two experiments which were conducted to investigate the effect of the acidified drinking water on growth performance in meat ducks from 1 to 35 d of age. The composition and nutrition levels of diet in Exp 1 and 2 keep the same. In Exp 1, 1200 one-day old ducks were randomly assigned to four treatments with fifteen replicates (20 ducks/ replicate). The pH values of drinking water with citric acid for four treatments were 6.90, 5.50, 4.50, and 3.50, respectively; In Exp 2, 140 one-day old ducks were randomly assigned to five treatments with seven replicates (4 ducks/ replicate). The pH values of drinking water with citric acid for five treatments were 7.80, 5.45, 4.45, 3.50, and 2.56, respectively. In Exp 1, body weight(BW), average daily gain(ADG), average feed intake (AFI) and average water intake (AWI) ducks aged from 1 to 35 d linearly decreased ($P<0.05$), and feed to gain ratio(F/G) increased in a linear manner by the decrease of the pH values in drinking water. In Exp 2, BW and ADG of ducks aged from 1 to 35 d were decreased ($P<0.05$) in a linear manner by the increment of the citric acid levels. Duck drank water with 2.56 pH value had the lowest water intake and had the highest F/G or feed to water ratio compared to the other four treatments($P<0.05$). In summary, the acidified drinking water for meat duck aged from 1 to 35 d has a negative effect on the growth performance. Low pH value in drinking water for a long time could inhibit the water intake in meat duck.

Keywords: pH value, drinking water, meat duck

S1- 0388 Effect of different protein restriction on performance, reproductive performance and egg quality of broiler breeders S3 line

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This experiment was conducted to evaluate the effects of different protein restriction on performance, reproductive performance egg quality of Broiler Breeders. Two hundred and forty hens of 25 weeks broiler breeders S3 line with similar body weight and laying rate were randomly divided into 4 groups with 6 replications in each group and 10 hens in each replicate, which were fed corn-soybean meal type of basal diet with 16.64% protein, the other 3 groups the same energy but different protein levels, were 17.64%, 15.64% and 14.64% respectively. The whole experimental period is 40 weeks. The results show that: 1) compared with the control group, the egg laying rate and the daily egg mass in low protein groups and protein group were found to be significantly lower ($P<0.05$), but the death and culling rate in low protein groups were significantly lower, and the laying performance in the low protein group 1 were significantly higher than tall protein group in late period of laying. 2) There were significantly different increase in reproductive performance by comparing the low groups and the control group and tall protein group in the early period of laying ($P<0.05$), compared with the control group the fertilized rate of the low groups increased 9.7% and 7.9%, hatchability increased 15.8% and 20.1%, and embryonic death reduced 26.1% and 24.3% respectively. 3) in the period of egg laying, the egg-shell strength of the low groups were significantly higher than the control group and tall protein group, there were different degrees in the egg yolk weight and albumen weight in the early or late period of laying ($P<0.05$). Therefore, low protein restriction can improve the ability of productive performance, reproductive performance and egg quality of broiler breeders, particularly for the protein level 15.64% in diet. These findings further advance our understanding of health production in broiler breeders.

Keywords: low protein limited feeding; broiler breeders; productive performance; reproductive performance; egg quality

S1- 0390 Effect of dietary complex mineral supplementation on performance of laying hens

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This experiment was conducted to investigate the effect of dietary addition of complex mineral (CM) on the performance, egg qualities, blood properties and yolk fatty acid composition of laying hens. At 20 weeks of age, a total of 450 Brown Nick were divided into five treatments with five replicates in each treatment. Five levels of complex mineral (0, 0.025, 0.050, 0.100, 0.200) were added with commercial diets (ME 2800 kcal/kg, CP 16%) and fed from 20 to 35 weeks of age. Feed and water was provided ad libitum. Egg production and feed intake was registered regularly and feed conversion ratio (FCR) was calculated (g of feed/g of egg mass) weekly. Blood and breast meat was sampled and analyzed at the end of experiment. Egg production was increased and feed intake was decreased by the addition of 0.025% CM in the diet ($P < 0.05$). Therefore, FCR was significantly decreased in CM addition group compared with that of control ($P < 0.05$). Eggshell thickness showed significantly higher in 0.025% CM treatments than that of control group ($P < 0.05$). Blood cholesterol, neutral fat of bird fed control diets seemed to be higher than the CM treated groups, but the glucose was contrast between control and CM addition group. Muscular fatty acid composition was not affected by the addition of complex mineral in the diet. As the results, the 0.025% supplementation of complex mineral was the optimum levels in the diet to improve the performance and egg qualities of laying hen.

Keywords: complex mineral, egg production, egg quality, blood composition, laying hens

S1- 0391 Effects of Clostridium butyricum on growth performance and lipid metabolism of broiler chickens

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The objectives of this study were to assess the effects of Clostridium butyricum on growth performance and lipid metabolism of broilers. A total of 256 one-d-old male Arbor Acres broiler chicks with similar BW (43.34 ± 0.15 g) were randomly allocated into 2 treatments with 8 replicates in a complete randomized design. Broilers were fed corn-soybean meal based diets and supplemented with 0 or 1×10^9 cfu of Clostridium butyricum/kg of diet for 42 days. Body weight and feed intake of broilers of each cage were measured on the morning of days 21 and 42 for determination of average daily gain (ADG), average daily feed intake (ADFI) and feed conversion rate (FCR). Mortalities and health status were visually observed and recorded daily throughout the entire experimental period. Blood samples from 8 birds (1 bird per cage) were obtained from each treatment on days 21 and 42 of the experiment to determine the serum hormones levels and biochemical parameters. All broilers appeared healthy throughout the entire experimental period. Birds in the Clostridium butyricum- supplemented groups had higher ($P < 0.05$) ADG and lower ($P < 0.05$) FCR during the grower phase and the entire period of the experiment. Besides, birds supplemented with Clostridium butyricum had higher ($P < 0.05$) insulin level in the serum at 21 day of age and higher ($P < 0.05$) growth hormone in the serum at both 21 and 42 days of age. However, leptin, free triiodothyronine and free thyroxine levels in the serum and concentrations of total cholesterol, triglyceride, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol in the serum at both 21 and 42 days of age were not significantly affected by the addition of Clostridium butyricum. The results of this study indicate that dietary supplementation of Clostridium butyricum improved growth performance and lipid metabolism of broilers.

Keywords: Clostridium butyricum, broiler, growth, lipid metabolism

S1-0392 Effects of enzymolytic soybean meal on growth performance, meat quality, intestine development and immune function of broiler

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This experiment was conducted to evaluate the effects of diet with different ESM (Enzymolytic soybean meal) concentration on performance, meat quality, intestine development and immune function in broilers, in order to detect the best additive amount of ESM in broiler diet. 432 1-day-old AA broilers were randomly assigned to 4 groups with 6 replicates of 18 birds each. Birds were fed basal diets supplemented with 0(control), 0.30%, 0.60% and 0.90% ESM for 42 days, respectively. The results showed as follow: as the increase of the ESM concentration in the diet, daily gain and FCR value of 42 days broiler decreased and showed a linear correlation ($P < 0.05$); as the increase of the ESM concentration in the diet, PH value and pectorales ratio respectively increase and decrease in a linear relationship ($P < 0.05$), while drip loss of pectorals decreased first and then increased which showed good conical relationship ($P < 0.05$); as the increase of the ESM concentration in the diet, thymus and bursa of fabricius rate in 42 days decreased first and then increased which showed good conical relationship ($P < 0.05$); muramidase activity in 21 and 42 days increased first and then decreased which showed good conical relationship ($P < 0.05$). The results showed that ESM have significant effects on performance, meat quality, intestine development and immune response in broiler. The appropriate dosage of compound acidifier in broiler diet for the optimal performance and inner environment is 0.60%.

Keywords: enzymolytic soybean meal, broiler, performance, intestine development, immune function

S1-0393 Influence of dietary inclusion of organic acids & plant extract mixture on broiler chickens growth performance, gastrointestinal development and health

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The aim of this study was to investigate the effects of dietary supplementation of a mixture of organic acids (short & medium chain fatty acids) and plant extracts on broiler chickens productivity, concentration of short chain fatty acids (SCFA), organ weights and histomorphometric parameters of duodenum and ileum and histopathology of the liver. Four hundred 1-d-old Ross 308 broiler chicks were randomly assigned to 2 dietary treatments for 5 weeks. The dietary treatments were 1) control diet, 2) diet supplemented with a mixture (Lumance® - from INNOV AD nv/sa, Belgium- a commercially available product that contains butyric, lauric, propionic acid and plant extracts) dosed at 0.5 g/kg of feed. The results showed that body weight, feed conversion ratio and SCFA profile were improved by the inclusion of this mixture compared with the control. The of 0.5 g/kg of the additive in broiler diets increased the intestinal weight (16.09 g), intestinal length (32.80 cm) and liver weight (29.31 g) versus the control ($P < 0.05$). In the experimental group on the 25th day the duodenum villus height decreased by 522.4 μm , but the ileum villus height increased by 81.8 μm versus the control ($P < 0.05$). On the 35th day, the duodenum crypt depth increased by 63.0 μm , the ileum crypt depth increased by 27.2 μm compared with the control ($P < 0.05$). In the analysed broilers liver samples, at 35th day of age, it was observed that 60% of samples did not have microscopic lesions, 40% with inflammatory cell infiltrate (lymphocytes, neutrophils) versus the control group, in which mention lesions compose of 40% and 60% respectively. In conclusion, a mixture of organic acids and plant extracts (0.5 g/kg of feed) can enhance broilers productivity, improve the development of the gastrointestinal tract, increase the production of SCFA and improve gut health.

Keywords: broilers, organic acids, growth performance, gastrointestinal health

S1-0394 Effect of L-selenomethionine supplementation during stress periods of starter broilers and chronic cyclic heat stressed finishing broilers

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Amongst different stress factors, the onset of broilers and the exposure of finishing broilers to heat have a significant impact on broiler production. The induction of oxidative stress by heat exposure is described in several papers as well the relation between selenium (Se) and oxidative stress. It is hypothesized that above normal feed Se levels, extra dietary supplementation with L-Selenomethionine could improve the performance of starter broilers and heat stressed finishing broilers. Two levels of added Se, 0 (control) and 0.2 mg/kg, in form of L-Selenomethionine (SeMet, supplied through the preparation Excential Selenium4000) were added to the basal starter (0-10d), grower (11-25d) and finisher diets (26-41d) containing respectively 0.5, 0.4 and 0.5 mg Se/kg. Diets contained 11.5, 10.5 and 9.5g Dig Lysine/kg respectively. Dietary treatments were replicated in 4 pens with 20 Ross308 birds each. A chronic cyclic heat stress model (T was increased to 34 °C for 7h, daily) was initiated at d28 of age. In the starter period (0-10d) a numerically difference between treatments was observed. Average daily gain (ADG) was 21.48 and 22.39 g/d and Feed conversion ratio (FCR) was 1.240 to 1.191 in control and SeMet supplemented group, respectively. In the finisher phase (26-41d) the supplementation with SeMet resulted in ADG of 90.72g/d versus 87.02 for control group. FCR was significantly improved by supplementation with SeMet from 2.380 to 2.043 ($P<0.05$). Overall (0-41d) results showed FCR of 1.891 and 1.722 and ADG of 63.50 to 64.99 in control and SeMet supplemented treatment, respectively. Overall mortality was 3.75 and 2.50%. In conclusion, supplementation of broiler diets with L-Selenomethionine could be a nutritional tool to optimize broiler performance during stressful periods, specifically during heat stress in finishing broilers.

Keywords: selenium, heat stress, broilers

S1-0395 Effect of different levels of organic acids on performance, blood parameters and gastrointestinal tract function of laying hens

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The aim of the present study was to investigate the effect of different levels of organic acids on laying hens' performance, blood parameters and gastrointestinal tract function. For the period of 8 weeks, 36 Hisex Brown laying hens were randomly assigned to 3 dietary treatments: 1) control diet, 2) diet supplemented with the mixture of organic acids (medium chain fatty acids, butyrate, essential oils and *Saccharomyces cerevisiae* (Lumance®)) at the level of 1 g/kg of feed, 3) diet supplemented with the same mixture as in treatment 2, but at a higher amount (2 g/kg of feed). All laying hens were kept under the same conditions. The indices determined in blood serum: total protein, bilirubin, cholesterol, DTL- and MTL- cholesterol, triglycerides, glucose, GGT, GOT, GPT, alkaline phosphatase, alpha amylase, contents of c-reactive protein, uric acid and lipase. Development of intestines and internal organs (intestinal length, intestinal weight, weight of glandular and muscular stomach, pancreas, heart and liver) were determined. The concentration of short chain fatty acids (SCFA) in caecal content was measured using method of HPLC. The results of the present study showed that neither 1 g/kg nor 2 g/kg supplementation of feed additive affected egg production, feed conversion ratio for the production of 1 kg egg mass, intestinal development and organ weights. Dietary supplementation of analysed additive in diets at the rate of 1 g/kg increased the amount of GGT by 55% and decreased uric acid by 67% compared to the control group ($P<0.05$). No significant difference in others blood indices between the treatments was observed. In experimental groups SCFA in caecum of laying decreased by 21-39% compared with the control group ($P<0.05$). Supplementation of different levels of organic acids had no significant impact on the laying hens' performance, gastrointestinal tract function and most blood indices.

Keywords: laying hens, organic acids, blood parameters, digestive tract

S1- 0396 N- acetyl- L- cysteine improves performance of chronic cyclic heat stressed finishing broilers

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Heat exposure has a significant impact on well-being and production of finishing broilers. The involvement of heat stress in the occurrence of oxidative stress has been described repeatedly. The most abundant endogenous intracellular antioxidant is the tripeptide glutathione, for which cysteine is the rate limiting amino acid. Therefore, it was hypothesized that dietary supplementation of N-acetyl-L-cysteine, as a source of cysteine, could improve the performance of heat stressed finishing broilers. Four levels of N- acetyl- L- cysteine; 0 (control), 500, 1000 and 2000 mg/kg, were added to a basal finisher diet with a ratio digestible M+C to digestible LYS of 0.73 (d25- 41 of age). Dietary treatments were replicated in 8- 9 pens with 20 male Ross308 birds each. A chronic cyclic heat stress model (T was increased to 34 °C for 7h, daily) was initiated at d28 of age. Rectal temperature at d41 was >0.2 °C lower for supplemented broilers as compared to control ($P > 0.05$). Final BW, growth and feed conversion in the finisher phase were all substantially and significantly improved ($P < 0.05$). ADG was 88.2, 92.2; 93.7 and 97.7 g/d, and the feed: growth ratio equalled 2.21, 1.91, 1.84 and 1.80 for the 0, 500, 1000 and 2000 mg/kg N-acetyl-L-cysteine treatments, respectively. In opposite, feed intake on the treatments 500, 1000 and 2000 mg/kg N-acetyl-L-cysteine was reduced by 10- 12%, as compared to control ($P < 0.05$); corroborating previous studies showing reductions in feed intake by excess of dietary cysteine. Mortality was not affected. In conclusion, N-acetyl-L-cysteine improved dose-dependently growth and feed conversion, but reduced feed intake, in heat stressed finishing broilers. Determination of more physiological endpoints will give more insight in the mechanisms of performance enhancement.

Keywords: N- acetyl- L- cysteine, sulfur amino acids, heat stress, finishing broilers

S1- 0397 Effect of a Buttiauxella sp phytase on sodium and amino acid digestibility in young broilers

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Previous work demonstrated that phytate interacts with pepsin and can increase HCl secretion in the gastrointestinal tract of monogastric animals. This is associated with increased NaHCO_3 secretion in the small intestine, and results in a negative impact on sodium digestibility. This could impact the function of Na-K pumps, and potentially have a negative impact on amino acid (AA) digestibility. The objective was to test the effect of a Buttiauxella phytase on sodium and AA digestibility in 21d old male broilers in two studies. Both studies shared the same design: treatments (6 replicates/treatment and 16 male Ross 308 birds/replicate) were a negative control (NC) diet with 2900 kcal/kg AMEn, 21% CP, 0.18% retainable P, 0.25% phytate-P, and 0.65% Ca, 4 graded levels of phytase over the negative control (250, 500, 750, 1000 FTU/kg), and 3 graded levels of supplemental MCP (0.06, 0.12, 0.18% P). Pelleted feed and water were available ad libitum for the whole experimental period (5-21 days of age). Performance was measured at d21 and tibias collected to determine ash content of 4 birds/replicate. Ileal digestibility of Na, P, Ca and AA was determined at day 21, using TiO_2 as the marker. Data from both trials were pooled for statistical analysis (JMP 11.0); trial was used as a random factor. Increasing phytase dose from 0 to 1000 FTU/kg feed linearly reduced FCR and increased BWG, FI, and ileal digestibility of Na, P, Ca, CP and AA ($P < 0.001$). A linear correlation was found between ileal Na digestibility and ileal AA digestibility ($P < 0.0001$). Phytase at 750 and 1000 FTU/kg increased ileal Na and mean AA digestibility vs MCP supplemented diets. At 1000 FTU/kg, Buttiauxella phytase resulted in similar BWG, FCR and tibia ash to the NC+0.18% P diet. Increasing phytase dose degrades more phytate, alleviating the anti-nutritional effect and increasing AA digestibility, which could be driven through increased Na-K pump activity as a result of increased ileal Na digestibility.

Keywords: broilers, Buttiauxella phytase, sodium, amino acid, digestibility

S1-0398 Chemical composition study of standard chicken serum

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Sterile filtered chicken serum was purchased from “Sigma-Aldrich” and studied using X-ray photoelectron spectroscopy in order to reveal chemical composition and distribution of the predominant elements. The main peaks observed in survey spectra were of C 1s (76.94 %), N 1s (3.73 %) and O 1s (13.47 %) core levels. There have also been observed small amounts of P 2p (0.96 %), Cl 2p (1.79 %), and Na 1s (3.11 %). The study revealed that carbon spectrum contains four components with the corresponding chemical bonds: C – C, C-N/C-OH, C=N/N-C=C, and C=O. Nitrogen ions were present in three forms: imine, amine and positively charged nitrogen. Oxygen ions were bonded with carbon and nitrogen, and were connected with water molecules. The obtained spectroscopic data contains considerable variety of elements and is similar in part to the bovine serum albumin fraction V and human albumin.

Keywords: chicken serum, chemical composition, spectroscopy

S1-0399 Dietary resveratrol supplementation protects against heat-stress-impaired growth performance and meat quality of broilers possibly through a mitochondrial pathway

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The naturally occurring polyphenol resveratrol (Res) has been acknowledged with many beneficial biological effects. This experiment was to evaluate the effect of dietary Res supplementation on growth performance, meat quality, and the mRNA expression levels of mitochondrial biogenesis-related genes and mitochondrial DNA (mtDNA) copy numbers in the breast muscle of broilers exposed to heat stress (HS). A total of 240 21-day-old male Cobb broiler chickens were randomly assigned to 3 different treatment groups, with 6 replicates of 15 birds each. The 3 treatment groups were as follows: the control (Con) group, in which chickens were housed at 22 ± 1 °C and fed the basal diet, and the HS and HS + Res groups, in which chickens were housed at 33 ± 1 °C for 10 h (0800-1800 h) and 22 ± 1 °C for the rest time and fed the basal diet with 0 and 400 mg/kg Res, respectively. The treatment lasted for 21 days. Compared with chickens in Con group, chickens in HS group not only exhibited lower average daily intake, average daily gain, body weight at slaughter, pH24h, mtDNA copy numbers, and the mRNA expression levels of peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α), nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (Tfam) but also exhibited greater L*45min, L*24h, drip loss, and heat shock protein 70 (HSP70) mRNA level ($P < 0.05$). Compared with chickens in HS group, chickens in HS + Res group not only exhibited lower L*24h, drip loss and HSP70 mRNA level but also exhibited greater body weight at slaughter, a*45min, a*24h, pH24h, mtDNA copy numbers, and the mRNA expression levels of PGC-1 α and NRF1 ($P < 0.05$). In conclusion, these results suggest that resveratrol is an effective feed additive to protect against heat-stress-impaired growth performance and meat quality of broilers, and the underlying mechanism may be partly due to the improved mitochondrial biogenesis induced by resveratrol.

Keywords: broiler, resveratrol, heat stress, meat quality, mitochondrial

S1-0400 The effect of dietary tryptophan and valine level in a low crude protein diet on production performance of laying hens

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Aim was to test the effect of a low crude protein (CP) diet with added L-tryptophan (Trp) and L-valine (Val) on production performance of Bovans Brown laying hens from 28 to 39 weeks of age. The trial was performed with five treatments and twelve replicates with twelve birds per replicate. Treatment 1 (positive control, PC) received a diet with 16.2% CP and a digestible Trp- to- digestible lysine ratio (dTrp/dLys) of 25% and digestible Val-to-digestible lysine ratio (dVal/dLys) of 100%. Treatment 2 (negative control, NC) received a diet with 13.5% CP and a dTrp/dLys of 19% and dVal/dLys ratio of 79%. Treatments 3 and 4 received diets with added L-Trp and L-Val, respectively, to a dTrp/dLys and dVal/dLys ratio comparable to the PC diet. For Treatment 5 the diet was added with both L-Trp and L-Val to dTrp/dLys and dVal/dLys ratios comparable to the PC diet. Measured parameters were feed intake, laying rate, egg weight, egg mass and feed conversion ratio (FCR). During the first weeks, feed intake was that low that birds were not able to increase laying rate and egg weight. Birds had to recover thereafter. Trial weeks 9-12 were representative for the effect of dietary Trp and Val levels on performance. Addition of L-Val to the NC diet resulted in a higher egg weight compared to the NC treatment ($P < 0.05$) and numerically higher than the PC treatment. Addition of L-Trp to the NC diet resulted in the highest laying rate and highest egg mass, resulting the lowest FCR after the PC treatment ($P < 0.05$). Addition of both amino acids to the NC diet was less effective and performance was only slightly improved compared to the NC treatment. In conclusion, this trial indicates that CP in layer diets can be significantly reduced without affecting production performance if daily intake of amino acids is covered.

Keywords: tryptophan, valine, requirement, ratio, laying hen

S1- 0401 Feeding a prestarter has a positive effect on male broiler performances

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The objective of the trial was to determine the effect of a prestarter on the production performances of male broilers. For this, one-day-old chicks were fed a prestarter (P, mash/pellet mixture) or a common starter (S, mash) on a carton plate. 12 pens (=replicates) with 30 male broilers per pen received either P or S (30 g/chick). After 24h the feed mangers with the starter feed were placed in the pens. A three-phase feeding scheme was applied with a starter (0-13d, mash), grower (13-26d, pellet) and finisher (26-36d, pellet) diet. Per pen, body weight (BW) and feed intake (FI) were measured, while weight gain (BWG) and feed conversion ratio (FCR) were calculated. In the starter and grower period, significant differences were found. At the end of the trial (0-36d), P fed broilers had a 3.9% higher mean BW (2602 g vs 2504 g), a 4.0% higher BWG (72.3 g vs 69.5 g) and a 2.8% higher FI (109.3 g vs 106.3 g) compared to the S fed broilers (all significant, $P < 0.01$), and their weight adjusted FCR 2500 was almost 4 points better (1.493 vs 1.529; significant, $P < 0.02$). It can be concluded that feeding 30 g prestarter per chick at arrival in the stable, significantly improves male broiler performances.

Keywords: broiler, prestarter, performances

S1-0402 Precision feeding layer pullets: how does it affect feeding behaviour?

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Social concerns about laying hen welfare in group housed systems stress the need for knowledge about the requirements for the individual animal. The effect of ad libitum or restricted feeding on feeding behaviour was studied in layer pullets. It was hypothesized that restricted birds would have reduced growth, would have a higher feed efficiency and would be more eager to eat than ad libitum fed birds. At hatch, thirty Lohmann brown layer pullets were allocated in a pen with a precision feeding station. Birds were trained to use the station from 0 to 24 d of age. At d 24, birds received an RFID tag and were randomly assigned to one of two treatments, ADLIB: no upper BW control, or RESTR: Lohmann brown target BW as an upper limit. From d 24 onwards, individual BW and feed intake were recorded at each visit to the station. Birds on the ADLIB treatment were provided access to a limited amount of feed for a short duration each time they visited the station. In the RESTR treatment, birds that met or exceeded the target BW were gently ejected from the station; birds that were under the target body weight were allowed to eat. All results were reported for 84 d of age. BW and cumulative feed intake was higher in the ADLIB treatment compared to the RESTR treatment (1156 vs 1039 g, $P = 0.001$; 3160 vs 2807 g, $P < 0.001$). Residual energy intake was 16.6 kcal/day higher in the ADLIB treatment compared to the RESTR treatment ($P = 0.001$). In addition, birds in the RESTR treatment ate more per meal (7.0 vs 5.5 g, $P < 0.017$) and had a higher daily visit frequency (44 vs 12 times per day, $P < 0.001$) than birds in the ADLIB treatment. RESTR birds entered the station more often when the room light was off than ADLIB birds (10 vs 2 times per dark period, $P = 0.001$). It can be concluded that birds on the RESTR treatment used their nutrients more efficiently than birds on the ADLIB treatment, that RESTR birds were more motivated to visit the station, and therefore more eager to eat.

Keywords: precision feeding, rearing, feeding motivation, laying hens

S1- 0403 Apparent digestibility and metabolizable energy value of a partially and a totally defatted black soldier fly meal in broiler chickens

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Aim of this study was to determine the apparent digestibility coefficients (ADC) and the apparent metabolizable energy (AME) of a partially and a totally defatted black soldier fly meal (BSFp and BSFt, respectively) in broiler chickens using total collection of feces. A basal diet was formulated and two assay diets were developed by substituting 250 g/kg (w/w) of the basal diet with BSFp or BSFt. One-d-old male broiler chickens (Ross 308) were raised in floor pens until 21 d of age. On day 21, 60 birds were randomly assigned to 15 cages (4 birds/cage). The birds were offered a commercial broiler diet until the introduction of the assay diets. On day 26, 5 replicate cages were randomly assigned to each assay diet. The digestibility was evaluated from 31 to 34 d. Data were tested by independent samples Student's t-tests. The ADC for dry matter (DM), organic matter (OM), crude protein (CP), crude fat (CF), gross energy (GE) and AME of BSF meals were calculated. Correction for zero nitrogen (N) retention was made to estimate the N-corrected AME (AMEn). The ADC of DM, OM, CF and GE were higher for BSFp than BSFt ($P < 0.05$). No significant differences were found for ADC-CP. The AME value was higher in BSFp than BSFt (14.07 and 11.85 MJ/kg, respectively; $P < 0.05$). The same trend was observed for AMEn (11.44 and 11.16 MJ/kg, respectively; $P < 0.05$). This study provides updated ADC of BSFp and BSFt. The acquired knowledge of AME and AMEn will be useful for nutritionists and feed companies.

Keywords: broiler chicken, digestibility, metabolizable energy, black soldier fly, insect

S1- 0404 Combination of cassava flour and abattoir blood as one ingredient (cassablood) in broiler ration

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A study was carried out to investigate the response of broiler chicken to a feed ingredient code-named "cassablood" which was developed from blended and dried mixture of cattle blood and cassava flour. 120 four weeks-old Zartech broiler chicks were randomly assigned to four dietary treatments containing cassablood at 0, 20%, 25% and 30% inclusion levels which constituted treatments 1, 2, 3 and 4 respectively, in a completely randomized design experiment. The effect of the dietary treatments on growth performance and economic indices of the broiler production was determined. Results showed that treatment effect on final body weight, final weight gain, feed intake and feed conversion ratio were significant ($P < 0.05$). Birds fed cassablood-based diets compared favourably with the control diet. 20% cassablood inclusion performed equally with the control diet in feed intake, body weight gain and feed conversion ratio but significantly better than 25% and 30% inclusion levels. Similarly, 20% and 25% inclusion level produced significantly ($P < 0.05$) best economic gains on feed cost per kilogram of broiler among all the treatments even though all cassablood-based diets were significantly ($P < 0.05$) cheaper than the control in producing one broiler chicken. Therefore, 20%-25% cassablood inclusion level is recommended in broiler diet for optimal production and profit maximization.

Keywords: cassablood, broilers, growth, economics

S1- 0405 Effects of dietary timing and density on performance of broilers

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The aim of this study was to investigate the effects of dietary timing (starter duration of one, two or three weeks) and dietary density with constant ratio of energy and protein (strain recommendation and 5 percent higher and lower) on male and female Cobb broilers in 42 and 49 days rearing period. Five hundred and four male and female Cobb broilers were used in a $2 \times 3 \times 3$ factorial experiment based on completely randomized design. The results indicated that sex had significant effects on weight gain, feed intake, feed conversion ratio, feed cost per kilogram weight gain and income over feed cost ($P < 0.01$). Timing of starter had significant effect on weight gain, feed cost per kilogram weight gain and income over feed cost ($P < 0.05$). Diet density had significant effect on weight gain, feed conversion ratio and feed cost per kilogram weight gain at 42 and 49 days of age. In conclusion, the results indicating that timing of starter have limited effect in longer terms and diluted diets can be used in 49 days rearing method.

Keywords: phase feeding, diet density, broiler, performance, economic

S1- 0406 How conventionally- and precision- fed broiler breeder hens use energy

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The effect of feeding systems was assessed on maintenance metabolizable energy (ME_m), carcass traits and partition of metabolizable energy intake (MEI) toward egg production from 23 to 34 week (wk) of age. A total of 480 Ross 308 broiler breeders were randomly assigned to two treatments precision feeding (PF) and conventional daily restricted feeding (CON) in 16 pens of 30 birds each. The PF system weighed birds when they entered to the station and allowed access to feed for 60 seconds if their BW was less than the target BW. The PF birds were fed by the station at any time whereas CON birds were fed daily every morning. Fat pad weight (FPW) and breast muscle weight (BMW) as a percentage of body weight (BW) were calculated for both treatments. The CON and PF treatments respectively, partitioned MEI toward egg production was 6% and 5.6% of ($P<0.0001$); the ME_m was 314 and 303 kcal/d ($P<0.0001$); the FTW was 2.03% and 1.76% ($P=0.005$) for the entire experimental period and BMW was 20.6% and 19.42% at wk 23 ($P=0.0114$). The CON birds reared in skip-a-day method (feeding on alternate days) in rearing period from 10 to 23 wk of age. Therefore, CON birds diverted more energy toward storage in abdominal fat pad (more efficient in storage and mobilization) due to conditioned to repeated energy shortage. Larger feed allocations to CON birds resulted in higher MEI, higher ME_m and greater BW compared to PF birds. Higher BW increase in CON birds reflected higher percentage of FPW compared to PF birds. Thus, CON birds were able to partition more energy toward egg production compared to PF birds. However, PF birds were reared on PF method in rearing period and they had always nutrients available from the gut which partitioned more energy toward maintenance of BMW at the start of laying phase. Moreover, the high maintenance requirement of the breast muscle in PF birds, in combination with the lower MEI prevented the energy excess needed to start the onset of laying.

Keywords: precision feeding, metabolizable energy intake, carcass traits

S1-0407 High inclusion levels of canola meal in poultry diets

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Canola meal (CM) is a widely used protein supplement in poultry diets. Dietary inclusion levels of CM have traditionally been limited due to the high content of fiber and the presence of anti-nutritional factors, including glucosinolates (GLS). Over the years, the content of GLS in CM has been declining steadily, and it is now ~4.0 µmol/g. In the current study, standardised ileal digestible (SID) amino acids and AMEn contents were determined with broiler chickens and turkeys. The AMEn and SID values for total amino acids averaged 1886 and 2088 kcal/kg, and 81.8 and 78%, respectively, for broilers and turkeys. The AMEn values increased to 1955 and 2196 kcal/kg for broilers and turkeys, respectively, following enzyme (multi-carbohydrase) supplementation. In the subsequent studies with broilers, turkeys, and laying hens, the effects of high dietary levels of CM on performance were evaluated. In the broiler study (1-35 d of age), BW gain and FCR averaged 2.32 and 2.30 kg, and 1.53 and 1.51 for the SBM Control and the diet containing 15% of CM, respectively. In the turkey trial (1-56 d of age), dietary inclusion of 20% of CM resulted in similar BW gain (3.75 vs. 3.90 kg) and FCR (1.73 vs. 1.71) values as those for the SBM Control diet. Diets containing 0, 4, 8, 12, 16 or 20% of CM were also fed to laying hens throughout the 24-week study. There were no significant differences in hen-day production, feed intake, feed efficiency, mortality, and egg quality between dietary treatments. The egg mass was not affected as a result of the same or better hen-day egg production in hens consuming CM diets. It could be concluded that CM could be used effectively at 15-20% of broiler chicken, turkey, and laying hen diets, providing the diets are formulated based on digestible amino acids and available energy contents. It could also be concluded that due to the low GLS content, high inclusion levels of CM would not adversely affect animal health and growth.

Keywords: canola meal, dietary level, broiler chicken, turkey, laying hen, enzyme

S1- 0408 Compositional characteristics of treated date pits and its utilization as a feed additive in broiler rations

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The objective of this study was to evaluate growth performance of broilers when fed diets containing fungi degraded (treated) date pits. *Trichoderma reesei* treated date pits were evaluated for their chemical composition and utilized as a feed additive in broiler rations. In the *T. reesei* treated date pits, total carbohydrate content was increased from (3.2% to 7.9 %) when compared to untreated date pits. The monosaccharide composition by HPLC analysis showed that the mannose content of the cellulose, hemicellulose and lignin fraction of untreated date pits is (14.7 %, 11.7% and 0.76%). After treatment with *T. reesei*, the concentration of mannose gets increased to (20.5%, 18.6% and 1.9%) respectively. Preliminary studies at our College, have shown that the addition of treated date pits to broiler diets significantly reduced the colonization of pathogenic bacteria (*Salmonella* spp, *Campylobacter* spp, *Shigella* spp and *Escherichia coli*) in poultry. The results showed that degraded date pits can be used as a natural growth promoter. Patents were granted in regards to this discovery by the EU and the US Patent and Trademark Offices. In this present study, one-day-old Cobb 500 chicks were randomly divided into 12 cages and fed to four different dietary treatments. Broilers in group 1 received corn-soy diet, while broilers in group 2 received corn-soy diet in addition to Oxytetracycline antibiotic. Besides broilers in group 3 were fed non-degraded date pits added to the corn-soy. In group 4, the broilers were fed degraded date pits added with the same percentages as in treatments 3 (10%). The body weight gain and the feed conversion ratio showed that there is no significant difference between all the treatments. However, the body weight gains were similar in both groups the degraded date pits and group 2 treatments and also were higher than the group 1 treatment. An overall, the results indicating a possible replacement for part of the corn-soy by date pits in broiler feed

Keywords: chemical composition, date pits, growth performance

S1- 0409 Effects of *Bacillus Subtilis* on growth performance, digestive enzymes, intestinal morphology, and colorectal microbiota of pigeon squabs

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The experiment was conducted to study the effects of *Bacillus Subtilis* on growth performance, digestive enzymes, intestinal morphology, and colorectal microbiota of pigeon squabs. A total of 540 pairs of parent White King pigeons were randomly assigned to 4 dietary treatments groups (control group, T1 group, T2 group, and T3 group), each of which included 3 replications of 45 pairs (one pair per cage). Pigeons were fed a basal diet supplemented with 0 mg/kg, 200 mg/kg, 400 mg/kg, and 600 mg/kg *Bacillus Subtilis*, respectively. The results showed that, 1) The body weights of T2 and T3 were tended to increase, but they were not significantly. 2) The villus heights of duodenum, jejunum, and ileum in T2 were increased by 32.81%, 26.08%, and 28.63%, respectively, and crypt depth of jejunum was decreased by 20.46%. The villus heights of duodenum and ileum in T3 were increased by 24.33% and 20.46%, and crypt depths of jejunum and ileum were decreased by 21.82% and 11.94%. 4) In colorectal microbiota of T3 group, the *E. coli* number was decreased while the *Lactobacillus* number was increased significantly. In conclusion, the addition of *Bacillus Subtilis* in parental pigeon diet can notably promote the development of villus, and decrease the *E. coli* number and increase the *Lactobacillus* number in colorectal content of pigeon squabs. Therefore, *Bacillus Subtilis* was beneficial to the gut health of pigeon squabs.

Keywords: *Bacillus Subtilis*, pigeon squabs, small intestine, colorectal microbiota

S1- 0410 Metabolizable energy and true amino acid digestibility of soy-bean meal in Chinese Daheng broilers

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A growing interest in home-grown breeds for high quality meat meet consumers' demand and protect the excellent genetic resources. So two experiments were conducted to evaluate the energy values (AME, AMEn, TME, TMEn), and the true amino acid (AA) digestibility of different sources of soybean meal (SBM) in Chinese native chicken breeds Daheng broilers. In experiment 1, twelve different SBMs from Sichuan province were used to determine the AME, AMEn, TME, and TMEn by using conventional broilers in TMEn assay. In experiment 2, the precision-fed cecectomized Daheng broilers were used to measure the true AA digestibility of the twelve SBMs. In each trial, 48 18-week-old male Daheng broilers were randomly assigned to 6 treatments with 8 replicates of 1 chicken. The broilers were force fed by 2% of body weight. The diets were formulated as semi-purified diets containing 17% protein with the corn starch as the basal diet. The results of experiment 1 showed that analyzed nutritional composition of the SBM samples varied substantially. The TMEn of corn starch were uniform across the three repeated experiments, which was 3.524 Kcal/g. The AME, AMEn, TME, and TMEn of the twelve SBM samples ranged from 2.080 to 2.663, 1.923 to 2.496, 2.074 to 2.676, and 2.035 to 2.566 Kcal/g, respectively. Significant differences ($P<0.05$) were observed in metabolic energy of different sources SBM. Results of experiment 2 indicated that the coefficient of variation in AA contents of different SBM ranged from 6.36 to 10.94%. There were significant differences ($P<0.05$) among the true AA digestibility of SBM samples. The AA digestibility was found to range from 78.16 to 94.38%. The TMEn and true amino acid digestibility values of SBM could be used for the formulation of diets for Daheng broilers.

Keywords: metabolic energy, true amino acid digestibility, Daheng broilers

S1- 0411 Effect of macadamia nut cake inclusion on growth performance of pasture raised broiler chickens

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Evaluating novel feedstuffs is imperative to deal with highly variable price of conventional feedstuffs for animals. Macadamia nut cake (MNC) is a byproduct of oil processing industry which is rich in energy and protein. A study was conducted to determine the optimum level of MNC inclusion in pastured broiler diet without adverse effect on their growth performance. A total of 96 day-old Cobb 500 broiler chicks were randomly assigned to 8 floorless cages equally to one of 4 treatments: a) control, without MNC; b) 5% MNC c) 10% MNC and d) 15% MNC inclusion. The chickens were raised on their respective diets fed in mash form for 4 weeks on starter phase diet and for 3 more weeks on finisher phase diet. From 2 weeks, cages were moved on pasture. The feed intake and body weight of the birds was recorded weekly and used to determine average daily gain (ADG), average daily feed intake (ADFI) and gain to feed (G:F) ratio. Data obtained were analyzed using mix procedure of SAS and the differences of means were declared significant at $P<0.05$. In starter phase, ADG did not differ across the treatments while the G:F ratio was significantly higher ($P<0.05$) in 15% MNC inclusion. Notably, ADG, ADFI and G:F ratio did not show any significant difference across the treatments in finisher phase. Similarly, ADG, ADFI and G:F ratio did not show significant difference in the total period of the experiment either. However, a trend ($P=0.0523$) was observed in ADFI in total period with mean of 15% MNC inclusion being lower than the control (96 ± 2.0 vs 105 ± 2.0). The results show that MNC can be incorporated as high as 15% in the broiler diets without any adverse effect on their growth performance. Inclusion of MNC in broiler diets will not only reduce feed cost of broiler production but also support in sustainability of the macadamia nut producers as well as processors.

Keywords: broiler chicken, coproducts, macadamia nut cake, novel feedstuff, pastured chicken

S1- 0412 Study the appropriate amount of feed by force-fed in TME assay in Daheng broilers

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The objective of this experiment was to study the appropriate amount of feed by force-fed in TME assay in Daheng broilers. The effects of different force-fed levels on the AME, AMEn, TME, and TMEn values of a corn-soybean basal diet were studied, while the broiler body weight loss, nitrogen balance, and energy utilization were investigated. 40 18-week-old male Daheng broilers were completely randomized design divided into 5 groups, and 4 groups were precision-fed by 1.0%, 1.5%, 2.0%, 2.5% of body weight, while the other group received no feed as negative controls for the measurement of endogenous losses of metabolizable energy. The assay contained 48h empty time and 48h collection excreta time. The results showed that the different force-feed levels had no significant effect on body weight loss, total nitrogen retained, and energy utilization ($P>0.05$). The average AME, AMEn, TME, and TMEn in corn-soybean diet were 2.768, 2.868, 3.204, and 3.307 Kcal/g, respectively. There were significant differences ($P<0.05$) of metabolizable energy among different force-feed levels, but reached a stable level at 1.5% and 2.0% level, and variability at 2.0% treatment is lower than that of 1.5%. In conclusion, the 2.0% force-feed level is appropriate for Daheng broilers.

Keywords: force-feed level, metabolic energy, Daheng broilers

S1- 0413 Nitrogen corrected apparent metabolizable energy of macadamia nut cake for broiler chickens

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Competition among food, feed, and fuel lead to wide variation in price of conventional feedstuffs like corn, wheat and soybean used in broiler chicken diets. So, it's imperative to explore and evaluate novel feedstuffs to develop cost-effective and sustainable broiler chicken feeding program. Macadamia nut cake (MNC) is a co-product of oil extraction industry which has not been well studied yet. Thus its usefulness as an animal feedstuff is very limited. The objective of this study was to determine the nitrogen (N) corrected apparent metabolizable energy (AMEn) of MNC for broiler chickens at different ages. For that, 160 d-old broiler chicks were used to conduct two energy balance studies at diverse ages using corn-soybean based control diet and MNC as test ingredient. In study 1, two dietary treatments consisting of control (0% MNC) and 6% MNC (94% control diet + 6% MNC) were fed from 4 to 11 d of age. AMEn of MNC was determined by subtracting AMEn of control diet from AMEn of test diet. In study 2, four treatment diets including control diet (0% MNC); 3% MNC (97% control diet); 6% MNC (94% control diet); and 9% MNC (91% control diet) were fed with 91, 94, 97, 100% of ad libitum intake from 17 to 25 d of age. It was assumed that the differences in AMEn consumption was only due to MNC source. The MNC used in both experiments were from same source. Feed intake, body weight, energy intake, energy excretion, N intake, N excretion were determined and were used to determine AMEn, and AMEn intake in both studies. In study 2, AMEn intake was regressed against feed intake with the slope estimating AMEn of MNC. Regression equation used was $Y = 3,249.5x - 156.69$ ($P<0.0001$; SE of the slope = 200; $r^2 = 0.85$). The AMEn of MNC was found to be 3,492 and 3,249 kcal/kg in study 1 and 2, respectively. Considering the gross energy of MNC being 4498 kcal/kg, MNC is utilized efficiently by broiler chickens. Thus, MNC can be incorporated in broiler feed on routine basis.

Keywords: apparent metabolizable energy, broiler chicken, energy balance, macadamia nut

S1-0414 Additivity of values for metabolizable energy and true amino acid digestibility in mixed diets fed to Daheng broilers

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This experiment was conducted to determine whether the metabolizable energy and standardized amino acid digestibility in three mixed diets fed to Chinese Daheng broilers is better predicted. There were 96 conventional and cecectomized 18-week-old male Daheng broilers were prepared, respectively. One corn, three different sourced soybean meal (SBM) or rapeseed meal (RSM) were collected, and formulated for three diets: corn-SBM diet, corn-RSM diet, and corn-SBM-RSM diet. All the feeds and diets were evaluated the nitrogen corrected true metabolizable energy (TMEn) by using conventional broilers, and the true amino acid (AA) digestibility by using cecectomized broilers in precision-fed TMEn assay. In each batch, 48 chickens were randomly assigned to 6 treatments with 8 replicates of 1 chicken. A group of chickens were fasted and used to measure the endogenous losses of metabolizable energy and digestibility of amino acids. The results showed that: the TMEn of corn were uniform across the three repeated experiments, which was 3.425 Kcal/g, while there were no significant differences in true AA digestibility in corn except Arg, Ile, Thr, Asp, and Ser. As expected there were no significant differences between the observed and predicted for TMEn in these three mixed diets. The deviations between observed and predicted TMEn values were ranged from -1.29 to 0.02, which is in the acceptable range. The differences between the measured and the predicted true AA digestibility differed ($P < 0.05$) from -0.78 to 16.95. There were no differences between the predicted and the measured values for digestibility of corn-SBM ($P > 0.05$), except His, Phe, Glu. While except His, Cys in corn-rapeseed meal and Arg, His, Phe, Thr, Asp, Glu, Ser in corn-SBM-RSM, there were also no differences between the predicted and the measured values ($P > 0.05$). In conclusion, the TMEn values and true AA digestibility in three mixed diets fed to Chinese Daheng broilers were better predicted.

Keywords: additivity, metabolic energy, true amino acid digestibility, Daheng broilers

S1- 0415 Contents of soluble non-starch polysaccharide β -galactomannan in alternative legume and non-legume feed ingredients

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Alternative legume and non-legume feedstuffs are commonly used in poultry and swine feeds. These feedstuffs possess undesirable components like non-starch polysaccharides (NSP) as anti-nutritional factors. Soluble β -galactomannan (β -GM), a representative type of NSP in soybean meal in particular, has been proven an immune stressor whose level in feed is shown to be quantitatively correlated to feed-induced immune response (FIIR) that in turn compromises the performance and intestinal integrity in broilers. To analyze the level of β -GM in alternative feedstuffs, high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was used to measure the contents of β -GM in 3 legumes, 5 non-legume meals and 8 cereal grains and cereal grain by-products collected in Asia Pacific region. The results showed that the soluble contents of β -GM in alternative legumes ranged from 0.03 to 0.825 % (SD=0.45) with the highest in lupin and lowest in white peas. They ranged from 0.045 to 3.68 % (SD=1.61) in alternative non-legume meals with the highest in palm kernel meal and lowest in cotton seed meal (42%, CP), and from 0.03 to 0.36 % (SD=0.11) in alternative cereal grains and by-products with the highest in soft pollard and lowest in sorghum. The results are meaningful when calculating the soluble β -GM level in diets that utilize these alternative feed ingredients. Quantified soluble β -GM in diets may serve to estimate the severity of immune stress and performance damage that dietary β -GM inflicts on the animals, and to indicate if β -galactomannanase supplementation is needed in feed to alleviate the detrimental effects.

Keywords: β -Galactomannan, immune stress, production performance, intestinal integrity

S1- 0416 Bioavailability of zinc oxides in broilers

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The objective of the study was to evaluate zinc (Zn) bioavailability in three sources of Zn oxide (ZnO): two sources collected from the market (ZnO1, ZnO2) and a novel source (ZnHZ, HiZox[®]), using ZnSO4 monohydrate (ZnS) as a reference. A low-Zn basal diet was formulated in which plant feedstuffs were the only source of Zn (22 ppm). Twelve other diets were then prepared by adding to the basal diet 7, 14 or 21 ppm of Zn for each source. Nine male ROSS broilers per treatment were included and allocated in individual cages. Birds received one of the 13 experimental diets from d 5 to d 21 of age. At 21 d of age, a sample of blood was removed and the right tibia excised. Data were analysed by analysis of variance using the GLM procedure of SAS. Bioavailability was calculated from the slope ratio (covariate coefficient), compared to zinc sulfate. Results indicate a significant interaction between the source and the level of Zn ($P < 0.001$) on plasma and tibia ash Zn concentrations. Indeed, ZnHZ induced a 16 and 29% increase of plasma Zn compared to the other sources suggesting a higher digestive availability. In the same way, bone Zn concentration was 7, 12 and 26% higher in birds having received ZnHZ compared to those fed ZnS, ZnO2 and ZnO1 respectively. Corroborating the idea under which this better bioavailability may be linked to a faster disappearance of Zn from the lumen, total and soluble Zn concentrations in the gizzard and the small intestine were lower in birds fed the novel source compared to the others. This study confirmed that Zn bioavailability in ZnO is variable and dependent of its origin and its processing.

Keywords: broilers, zinc oxide, zinc bioavailability

S1-0417 The study of optimal lysine level in the diet of laying pigeon reared under high-temperature conditions

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The objective of this study was to evaluate the effect of the level of lysine in pigeon feed on performance, egg quality and serum biochemical indices, to discuss the appropriate lysine requirement. A total of 2400 healthy pigeons aged 40 weeks with similar body weight were randomly allocated to 6 groups with 5 replicates per group and 80 hens per replicate. The first group fed basal diet which was primarily composed of corn- soybean meal with low lysine level of 0.64%. The lysine levels of the other five groups were 0.68% , 0.72% , 0.76% , 0.80% and 0.84% for 12 weeks after a 1-week adaptive phase. The results showed: laying rate and average egg weight of whole experiment period were significantly influenced by lysine level of feed ($P < 0.05$), laying rate showed a significant quadratic response to increasing dietary lysine level ($P < 0.05$), the regression equation was $y = -86.7187x^2 + 136.2x - 45.6476$ ($R^2 = 0.882$, inflection point = 0.784), egg weight showed a significant linear response to increasing dietary lysine level ($P < 0.05$). Dietary lysine level had significant effects on eggshell thickness and yolk colour ($P < 0.05$), both showing significant linear response to increasing dietary lysine level ($P < 0.05$). Eggshell thickness showed a downward trend when dietary lysine level was increased, but yolk colour showed an opposite trend. Dietary lysine level had significant effects on the content of Ca, P, total cholesterol and total protein ($P < 0.05$). The content of Ca and total cholesterol showed significant linear responses ($P < 0.05$), Ca showed an upward trend when dietary lysine level was increased, and total cholesterol showed an opposite trend. The content of P showed a significant quadratic response ($P < 0.05$), the regression equation was $y = 13.2878x^2 - 16.4447x + 8.1641$ ($R^2 = 0.747$, inflection point = 0.619). In conclusion, the optimal lysine level of dietary was 0.78% under high-temperature conditions according to laying rate and feed to egg ratio.

Keywords: pigeon, lysine, performance, egg quality, serum biochemical indices

S1-0418 Effect of pawpaw leaf meal inclusion and exo- enzyme supplementation on performance and haematology of broiler chicken

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The effect of Pawpaw leaf meal (PLM) inclusion and enzyme supplementation on broiler chickens was evaluated in a 6-week feed trial. One hundred and twenty (120) arbor acre 14 day- old broilers were selected from a larger flock that was raised on commercial diet, balanced for weight and distributed into 12 pens. These pens were allotted to 4 dietary treatments randomly such that each experimental diet was fed to 3 replicates. The experiment was carried out in a 2x2 factorial arrangement comprising 2 enzyme levels (0 and 0.50 g/kg) and 2 PLM inclusion levels (0 & 5%). Thus, 4 experimental diets were formulated and designated as diet 1 (0%-E), diet 2 (0%+E); diet 3 (5%-E) and diet 4 (5%+E). Diets 1 and 3 were not enzyme supplemented but had PLM inclusion at 0 and 5% respectively, while diets 2 and 4 were enzyme supplemented at the rate of 0.5g/kg and had PLM inclusion at 0 and 5% respectively. Final live weight (2.48 kg/b) and total weight gain (2.11 kg/b) of broilers fed with 0.5 g/kg enzyme supplemented diets were higher ($P<0.01$) than those fed 0 g/kg enzyme supplemented diet. Also, PLM inclusion significantly ($P<0.05$) increased the total weight gain with a prediction equation $Y_{PLM} = 0.6056xTWG + 1.5728$; $R^2=0.76$; $P<0.02$. Enzyme supplementation led to significant increase ($P<0.05$) in MCV and decrease ($P<0.01$) in MCHC values of the broiler chickens; while PLM inclusion levels significantly ($P<0.01$) increased PCV. Enzyme and PLM was significant ($P<0.05$) for MCHC and platelets. High density lipoprotein was significantly ($P<0.02$) reduced by enzyme; while the AST was significantly ($P<0.05$) reduced by PLM levels. Enzyme x PLM interaction was significant ($P<0.05$) for cholesterol and LDL. In conclusion, PLM is a potential useful feed material for broiler chicken and it is suggested that 5% PLM could be used in broiler diet.

Keywords: pawpaw leaf meal, exo-enzyme broiler chicken performance haematology

S1- 0419 Effects of dietary nutrient density on semen quality and fertility of Jing Brown rooster

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The study was to investigate the effect of two different levels of dietary nutrient densities on semen volume, semen quality and fertility in breeder roosters. A total of 400 17-week-old Jing Brown roosters were randomly allocated into one of the 2 treatments. Each treatment consisted of 5 replicates of 40 roosters each. The control birds fed a layer diet containing 2670 kcal AME/kg, 15.9 % crude protein, and 3.64% calcium, while the experimental birds fed a rooster diet containing 2800 kcal/kg, 14.0 % crude protein, and 1.45% calcium. Semen samples were collected weekly continued for 8 weeks. Semen characteristics from both groups were evaluated. The results showed that the volume, sperm count, sperm motility and fertility of the experimental group were 0.52 ml, 4.41×10^9 sperms/ml, 83.54%, and 93.62%, respectively. Birds fed the rooster diet had higher semen volume (13.0%), sperm count (6.1%), fertility (0.8%) ($P < 0.05$) and higher sperm motility (5.7%) ($P < 0.01$) compared with the control. The results indicate that the rooster diet improved reproduction performance of the roosters.

Keywords: rooster, semen quality, fertility, Jing Brown

S1-0420 Protective effects of chicken egg yolk antibody against experimental *Shewanella marisflavi* infection in the sea cucumber (*Apostichopus japonicus*)

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Skin ulcer syndrome in sea cucumber is a bacterial infection disease with fast and high mortality. Yolk antibody (IgY), a kind of immune globulin, have the similar function as IgG. The cost of IgY is relatively low and is much easier for mass production, besides, IgY will not cause a series of problems as chemicals and antibiotics did, so IgY may become the next generation of biologic product to substitute for antibiotics. In this paper, we studied the protection of IgY to the skin ulcer syndrome in sea cucumber model induced by injecting intraperitoneally *Shewanella marisflavi*. Inactivated whole cell of *Shewanella marisflavi* was used as an antigen to immunize laying hens. The highest titer of the obtained specific yolk antibody by ELISA was 1:95000, and the high titer (>10000) could last for 13 weeks. The final purity of IgY after water dilution, two-step salt precipitation and ultrafiltration was 85.6%. The result of transmission electron microscopy and immunofluorescence experiment showed that specific yolk antibody can be combined with the *Shewanella marisflavi* specificity, and it can make bacteria agglutinated. The sea cucumbers treated with 0.5mg/g, 0.25mg/g and 0.05mg/g anti-*S.marisflavi* IgY(aSIgY) could achieve survival rates of 77.5%、47.5% and 22.5% at day 12 when the infection and injection therapy were carried out at the same time, respectively. However, the survival rates of sea cucumbers treated with 0.5mg/g non-specific IgY were only 12.5% at day 12. All sea cucumbers in saline-treated groups died within twelve days after bacterial inoculation; The sea cucumbers treated with 0.5mg/g aSIgY 1d and 3d after bacterial infection achieved survival rates of 52.5% and 22.5% respectively; The sea cucumbers treated with 0.5mg/g aSIgY 3d and 7d before bacterial infection achieved survival rates of 57.5% and 30% respectively. In conclusion, the study suggested that aSIgY has a positive protective effect for sea cucumber infected with *Shewanella marisflavi*.

Keywords: egg yolk immunoglobulin, *Shewanella marisflavi*, skin ulcer syndrome, sea cucumber

S1-0421 Effects of mannan oligosaccharide and Curcuma xanthorrhiza essential oil on stress indicators of broilers subjected to cyclic heat stress

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An experiment was intended to explore the growth performance and heat stress biomarkers of finishing broilers under cyclic heat stress (HS) as modulated by supplementation of mannan oligosaccharide (MOS) and Curcuma xanthorrhiza essential oil (CXEO). Two hundred forty 21-d-old Ross 308 male broilers were allocated in 4 experimental treatments, each of which was replicated 6 times with 10 broilers per replicate. The diets included a control (CON), CON + 5 g of MOS/kg, CON + 400 mg of CXEO/kg of feed, and 5 g of MOS/kg + 400 mg of CXEO/kg. From d 22 to 42, birds were subjected to cyclic heat stress by exposing them to 33° C for 10 h (from 0700 to 1700) and 22° C from 1700 to 0700. Body weight and average daily gain were significantly increased in birds fed MOS or MOS + CXEO diets. Compared with CON, dietary treatments reduced corticosterone and creatine kinase levels, heterophils to lymphocytes ratio, and mRNA levels of heat shock protein 70 in the muscle breast and jejunum. It was concluded that dietary supplementation of either MOS or CXEO alone or in combination may reduce some of the detrimental effects of HS in broilers.

Keywords: prebiotic, heat stress, broiler, Curcuma xanthorrhiza

S1- 0422 Effects of bee pollen and propolis on heat stress biomarkers in broilers subjected to high ambient temperature

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This study investigated the supplemental effects of bee pollen (BP) and propolis (Pro) on broiler performance, hematological profile, and biomarkers of heat stress in broilers reared under high ambient temperatures. A total of 240 one-day-old, male broiler chickens were divided into 4 treatment groups: control or supplemented with 20 g of BP/kg of feed, 3 g of Pro/kg of feed, 20 g of BP/kg + 3 g of Pro/kg. Results showed that dietary BP and Pro improved average daily gain and average daily feed intake of broilers particularly during the starter period ($P < 0.05$). Biomarkers of heat stress including the circulating heterophil-to-lymphocyte ratio, creatine kinase and mRNA expression of a 70 kD heat shock protein (HSP70) levels in breast muscle were decreased in supplemental birds. These findings suggest that dietary use of both BP and Pro as a feed additive may offer a practical nutritional strategy at high temperatures to prevail the harmful effects of heat stress in broiler production.

Keywords: bee pollen, propolis, broiler, heat stress, performance

S1-0423 The effect of crude protein intake on sperm quality of young and old male broiler breeders

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Continual genetic improvement in broiler growth traits necessitates careful management of the breeding stock to maintain fertility. In many production systems, male and female broiler breeders are both fed a female ration at different intakes calculated to allow adherence to the growth curve. While this may be of practical value, crude protein (CP) is one of the most costly components of the feed, and the nutrient requirement of males for protein is less. This experiment compared fertility of male broiler breeders with different CP intakes from 27 to 60 weeks of age (in 2 phases) and in a different group of birds from 52 to 60 weeks of age by assessing sperm concentration, sperm mobility and the number of points of hydrolysis in the inner-perivitelline layer (IPVL) of eggs from commercial egg-type hybrids after insemination to predict fertility. There was no response to CP intake observed in sperm concentration or sperm mobility. The predicted fertility of eggs increased slightly with an increase in CP intake in older birds but in each case only accounted for 1 to 10% of the variation in egg fertility and therefore should be viewed cautiously. The results indicate that there may be a positive effect of increasing CP intake as birds' age to improve fertility but that lower CP intakes have no negative effect on sperm concentration or mobility.

Keywords: cockerel, mobility, perivitelline membrane

S1- 0424 Efficacy of different anti-stressors on growth performance and immunity of broilers reared during hot and humid season

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Use of different anti stressors is a common practice in today's poultry industry to lower the negative impacts of stress. Present study was planned to compare the efficacy of different anti stressors on growth performance and antibody response against Newcastle Disease (ND) in broilers kept during hot and humid season. For this purpose, a total of 200 birds were procured from a commercial hatchery and randomly divided according to Completely Randomized Design in to 5 treatment groups {Control, Betain @ 2g/Kg, Chia (Salvia hispanica) @ 5g/Kg, Potassium Chloride @ 2g/Kg, and Vitamin C @ 2g/Kg} with 4 replicates of 10 birds each. Growth Performance [Feed intake (g), body weight (g), FCR, Performance number, Production index, Point spread, uniformity %) was recorded at the age of 6th week, while for antibody titer blood samples were collected at 7th, 17th, 36th and 42nd day. The data obtained were analyzed using Analysis of Variance (ANOVA) technique and significant means were compared using Duncan's Multiple Range test with the help of SAS 9.1. Results of the present study showed highest feed intake in birds supplemented with Chia, best FCR and highest body weight in birds supplemented with Betain. Whereas, non-significant differences were observed among all treatments for mortality (%), uniformity (%) and other performance parameters like Point spread, Production index, performance number. Furthermore, antibody titer against ND showed non-significant differences when compared at 7th and 17th day. But, at 35th and 42nd day the birds supplemented with Chia had significantly higher antibody titer against ND. Hence, it is concluded from the present study that Betain have better efficacy as anti-stressor.

Keywords: anti-stressor, Chia, betain, point spread, broiler

S1- 0425 Influence of Lactobacillus acidophilus LAP5 on gut health of broiler chickens

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Poultry gut health has become one of the most interesting fields of research in post-antibiotic era. The aim of the present study was to evaluate a probiotic strain, Lactobacillus acidophilus LAP5, as a replacement for growth promoter antibiotic supplementation in broiler's diet. LAP5 was isolated from the intestinal tract of a healthy pig and had great tolerance to gastric acid and bile salt, and the ability to adhere to the epithelial cell was significantly higher than other lactic acid bacteria ($P < 0.01$). It also showed great ability of adherence to crop epithelial cells and intestinal villi of chicken by using scanning electronic microscope and fluorescein isothiocyanate (FITC)-label method. In the competitive exclusion test, chickens were fed 2 mL of LAP5 for three days (10^9 cfu/mL) and were challenged with *S. typhimurium* E29 (10^7 cfu) at 4th and 5th day. The result showed LAP5 strain could reduce the invasion of Salmonella bacteria to liver and spleen 20-60% and 13-40%, respectively. Further, the effect of supplementation in diet of either LAP5 (10^9 CFU/g) or antibiotic (zinc bacitracin) on intestinal health and immune properties in 450 broilers were compared. Supplementation of LAP5 could reduce the cell count of Coliform in the crop and jejunum pH ($P < 0.05$), increase total volatility fatty acid in the ileum and lactic acid in the caecum ($P < 0.05$). The IgA and IgG titer in the jejunum and ileum, IFN- γ concentration in the serum were also increase in LAP5 group compared to the other groups. Base on the results of this study, LAP5 has excellent probiotic properties that could be used as a diet supplement for poultry.

Keywords: Lactic acid bacteria, Lactobacillus acidophilus LAP5, probiotic, post-antibiotic era, immunity

S1-0426 Effects of parental sex and bird nursing number on the nutritional components of pigeon milk and the growth performance of young squab

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In the current study, the nutritional components of pigeon milk and the growth performance of young squab under different parental sex and bird nursing number were investigated. A total of 288 squabs were sampled at post-hatching day 2, 4, 6, and 8 for evaluation of the pigeon milk, and another 36 squabs were tested throughout the whole 28 post-hatching growth day for evaluation of growth performance. The results showed that parental sex, bird nursing number and age in days significantly affected the nutrients in pigeon milk. Male parental pigeons secreted milk with more CP (crude protein), CF (crude fat), TE (total energy), P (phosphorus), and the amino acids Val, Gly and His compared to the female pigeons. In addition, lower amounts of the routine nutritional components (TE, CP, CF, P, Ca) and all the amino acids analyzed were detected as the bird nursing number increased from 1 to 2. Throughout the trial, the squabs fed by the male pigeons showed better growth performance than the squabs fed by the female pigeons. The female pigeons had a positive body weight change despite the large amounts of pigeon milk and feeding task output, while the male pigeons showed a negative change during the same period. In conclusion, the pigeon milk secreted by the male parent was higher in nutrients than that from the female and led to a better growth performance of the young squab. As the nursing number increased, diluted nutrients in the milk were received by the squabs, but caused no significant difference in growth performance. The nutrient differences in the milk might contribute to the differences in body deposition metabolism between the genders in parental pigeons. In designing the diet of parental pigeons during lactation or designing the diet for artificial feeding of squab, the nutrient parameters of milk from the male pigeon should be the preferred reference source.

Keywords: pigeon milk, parental sex, bird nursing number, nutritional component, squab growth performance

S1-0428 Effect of Chinese herb extracts for egg laying hens

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This experiment aims to study Effect of Chinese herb extracts for egg laying hens' production performance and egg quality. 3600 Hy-Line varieties can be divided into three groups, each group 1200. The Group B is experimental group and feed the basic complete diet pellets what add the traditional Chinese medicine extract. The group A is a control group and feed the basic complete diet pellets what add other medicine extract that is on the market in sales; The group C is control group and feed the common basic complete diet pellets. Results showed that: (1) Chinese medicine extract had no effect for laying hens on feed intake. (2) Chinese medicine extract can enhance the rate of laying hens. (3) Chinese medicine extract can improve egg weight and egg shell thickness, etc. The study showed that Chinese medicine extract can effectively improve the production performance of laying hens eggs and egg quality.

Keywords: Chinese herb extracts, laying hen, production performance, egg quality

S1- 0429 Effects of *Bacillus licheniformis* on production performance of commercial layers during heat stress

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In this study, the effect of B-Act®* on production performance of layers subjected to heat stress was investigated. B-Act® is a direct fed microbial intended to provide live microorganisms in which the active ingredient is a spray-dried spore forming bacterium *Bacillus licheniformis* with a minimum concentration of 3.2×10^9 viable spores/gram. Ninety six Babcock layers of 26 weeks old were raised in a standard open farm in India during summer time. Hens were housed per six in a California cage system and the average air temperature was 41°C. The layers were fed a commercial diet and divided in two groups. One group was supplemented with 0.5 kg B-Act® per ton of feed. Over a period of nine weeks egg production, egg weight and feed consumption was measured. Supplementing B-Act® increased egg production significantly ($P < 0.05$) with 4.4% resulting in an average laying rate of 98.4%. Although hens produced more eggs, egg weight remained equal (52.9 grams per egg). There was no difference in feed consumption between the control and the B-Act® group. However the feed conversion was numerically improved for the B-Act® supplemented layers. In conclusion, *Bacillus licheniformis* ameliorates egg production impaired by heat stress.

Keywords: *Bacillus licheniformis*, probiotic, laying rate, heat stress

S1- 0430 Effects of *Bacillus licheniformis* on performance, intestinal morphology and *Salmonella* colonization

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The aim of the present study was to evaluate the effect of B-Act® on performance, intestinal morphology and *Salmonella* colonization in broilers. B-Act® is a direct fed microbial intended to provide live microorganisms in which the active ingredient is spray-dried spore forming bacterium *Bacillus licheniformis* with a minimum concentration of 3.2×10^9 viable spores/gram. One hundred fifty thousand mixed sex Ross 308 broilers were housed in a standard farm in Thailand consisting of 10 houses. The broilers were fed a commercial diet and divided in two groups, 5 houses per group. One group was supplemented with 0.5 kg B-Act® per ton of feed. At 48 days old the B-Act® birds had a slaughter weight that was on average 6% heavier. Feed conversion ratio and mortality were respectively decreased with 5% and 2.34% versus the control group. Villi height was measured at the midpoint of the duodenum and 10 cm proximal of the ileocaecale junction of a representative number of broilers. Supplementing B-Act® to broilers resulted in a significant increase of villi height in the duodenum (141.6 µm or 13%) and in the ileum (261.3 µm or 42%). At slaughter, cloacal swabs of fifty broilers per treatment were analysed for *Salmonella*. In the control group 83% were positive while only in 60% of the B-Act® broilers *Salmonella* could be detected. Supplementing *Bacillus licheniformis* to broilers resulted in a higher absorption surface, less *Salmonella* and thus higher zootechnical performance.

Keywords: *Bacillus licheniformis*, probiotic, performance, *Salmonella*

S1-0431 Understanding the relationship of dietary metabolizable energy and ideal protein in modern broilers. 1. growth, carcass, metabolites, and hormonal profile

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The genetic improvement of broilers should lead us to constantly re-examine metabolizable energy (ME) and amino acids (AA) requirements of birds. Few recent studies and limitation in the design of many of the experiments have led to contradictory conclusions on the response of broilers to ME, AA and their interaction. In the present study, we evaluated this interaction at one growth stage (21 to 37d) addressing experimental shortfalls of previous investigations. A total of 2160 one-day-old Ross 308 male broilers were fed on commercial diets until 20 d of age. Thereafter, pellet corn-soybean meal based diets, were formulated based on 4 levels of true MEn (TMEn): 2875-, 3000-, 3125-, and 3250 kcal/kg by 3 levels of ideal protein (IP) (85%, 100%, and 115% of Evonik's AMINOChick®2.0). Each ME by IP combination had 9 replicates (20 birds/rep). TMEn, live performance, carcass parameters, and major blood metabolites and hormones were measured. Data were analyzed by 2-way ANOVA, using LSM method of JMP®. No interactions of ME by AA were found for any of the live-, carcass-, and metabolic parameters. Significant effects of dietary ME on feed intake (FI) and FCR were observed ($P<0.0001$), with the lowest FI and best FCR recorded with birds fed 3250 kcal/kg TMEn. IP also had an effect on FI ($P<0.0001$), however, birds fed levels beyond 100% showed no significant change in FI, though significant improvement in FCR ($P<0.0001$). This effect was further supported by significant improvements ($P<0.0001$) in carcass and breast meat yield, with the highest yield recorded with the 115% IP. On the contrary increasing ME lead to reduction in breast meat yield ($P<0.0001$). Blood concentrations of non-esterified fatty acids showed a significant response to increasing ME ($P<0.05$) while IP levels influenced T3 and T4 ($P<0.05$). Findings suggest under commercial nutritional dietary ranges broilers respond independently to ME and IP, and opportunities exist in increasing current recommendations of IP.

Keywords: broilers, metabolizable energy, amino acids, ideal protein

S1- 0432 Efficacy of butyrate and heptanoate dietary additives against Salmonella in broilers

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This trial evaluated the preventive effect of protected salts of organic acids against a *S. Enteritidis* infection. The effects were measured on fecal shedding and in organ contents, in broilers challenged with *S. Enteritidis*. A total of 160 one day old chickens were divided in four dietary treatments, with four pens of 40 birds each. Animal was the experimental unit. Treatments were: T1) Control feed, T2) T1 with 1.3 kg/t of Gustor N' RGY (70% sodium butyrate (NaB) protected with sodium salts of PFAD (palm fatty acids distillates)), T3) T1 with 3 kg/t of 30% NaB coated with vegetable fat, and T4) T1 with 1 kg/t of 65% sodium heptanoate protected with hydrogenated PFAD. At 5th day, 20% of the animals in each group were orally inoculated with 106 CFU of *S. Enteritidis* and the presence in faeces was evaluated in all animals with cloacal swaps on days 6, 12, 19, 26, 33 and 41. The animals were euthanised on d41, and crop, ceca, liver and spleen were sampled for microbiological counts. Analysis of *Salmonella* in faeces showed a decrease in the percentage of positive plates (from 85 to 40%, $P<0.05$) in supplemented treatments respect to the control at d41. However T4 on d12 and T2 on d19 did not improve T1 results. No presence of *Salmonella* was detected in liver. However, in the other organs the highest values were found for control group. In ceca, results were 94, 38, 56 and 19%, for T1, T2, T3 and T4, respectively ($P<0.05$). In crop results were 94, 19, 19 and 19%, for T1, T2, T3 and T4 ($P<0.05$). In spleen, only birds fed the control (75%) and the T4 feed (19%) showed *Salmonella* infection. Moreover, there was a tendency to prevent spleen infection between butyrate vs heptanoate. It can be concluded that butyrate and heptanoate feed additives reduce the presence of *Salmonella* in feces and are able to prevent systemic infection in broilers experimentally challenged with *S. Enteritidis*.

Keywords: feed additives, butyrate, broilers, salmonella enteritidis

S1- 0433 Effect of chlorogenic acid on growth performance, egg quality and serum antioxidant index in Hy-Line Brown layers

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This experiment was conducted to investigate the effects of chlorogenic acid on growth performance, egg quality and Serum antioxidant index in Hy-Line Brown layers. Using the same wks, uniformity of type, laying rate is 82% of hy line Brown laying hens 216, divided for three treatments, each treatment group of six, each repeat had twelve chickens, the control group was fed a basal diet, test group was fed with adding different proportion of chlorogenic acid diet (100 and 200 g/t chlorogenic acid) and test for six weeks. The results showed that: 1) The proportion of egg yolk in 200g/t dose group was higher than that of control group and significantly higher than that of 100g/t group. 2) dietary chlorogenic acid had significant effect on Hyline layers the average daily feed intake ($P < 0.05$). 200g/t dose group hens average daily feed intake was significantly lower than the control group. 3) diet add 100g of chlorogenic acid and 200g chlorogenic acid Hy-line layers of serum total antioxidant capacity (T-AOC) higher than that of the control group, but the difference was not significant. Diet add 100g of chlorogenic acid and 200g chlorogenic acid of Hyline layers of serum total superoxide dismutase (T-SOD) activity is higher than that of the control group, but the difference was not significant. Diet add 100g of chlorogenic acid and 200g chlorogenic acid of Hyline Brown laying chicken Qing malondialdehyde (MDA) content was lower than that in the control group but the difference was not significant. Comprehensive all indexes and diets adding chlorogenic acid to raise the proportion of Hyline Brown laying egg yolk and decrease of Hyline Brown laying average egg weight, improve the antioxidant ability of laying hens.

Keywords: chlorogenic acid, Hy-Line Brown layers, growth performance, egg quality, serum antioxidant index

S1- 0434 Effect of two extracts of plant flavonoids on performance of broilers fed corn- wheat- soybean meal-based diets

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The current study evaluated the effect of supplementation two different extracts of plant citroflavonoids, mostly naringine, extracted from bitter orange and grapefruit (Bioflavex[®] CA; BCA and Bioflavex[®], B) on performance of broiler chickens fed corn- wheat- soybean meal-based diets. Male Ross 308 day-old broiler chickens (1056) were randomly allotted to 4 treatments with 12 replicates pens/treatment and 22 birds/pen. The dietary treatments were; 1) a control basal diet; C, 2) C + 200 g/MT of BCA; BCA200, 3) C + 300 g/MT of BCA; BCA300, and 4) C + 500 g/MT of B; B500. The diets were fed in 2-phases from d 1 to 21 and 22 to 42. Body weight, feed intake, daily gain and feed efficiency were determined at day 1, 21 and 42 days. Data were analyzed as completely randomized design by GLM of SPSS v.19.0 and means separated using Tukey's test. Broilers receiving BCA200 grew 4.8% more than C from 1-21d, showing broilers on BCA300 and B500 intermediate values (35.3, 37.0, 36.8 and 36.5 g/d for C, BCA200, BCA300 and B500, respectively; $P = 0.0399$). Overall growth and gain to feed ratio were not affected ($P > 0.05$) by treatment. Mortality was numerically ($P > 0.05$) lower in BCA300 compared with C, BCA200 and B500. In conclusion, the current study showed that BCA inclusion at 200 g/MT significantly improved growth of broilers at 21 days of age. Thus, supplementation of broiler diets with Bioflavex CA might improve performance at early ages, at least until 21 days.

Keywords: flavonoids, broilers, performance

S1-0435 Effect of supplemental bacterial phytase at different dietary levels of phosphorus on tibial bone characteristics and body weight gain in broilers

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A 5- weeks feeding trial was carried out to determine the effectiveness of Bacterial Phytase (Phyzyme®) in broilers, at different dietary levels of Phosphorous. 140 d-old broilers (Hubbard) were randomly divided into 4 groups (n=4). Birds were fed corn based basal diet or the same diet supplemented with 3 different levels of non Phytate Phosphorous (NPP) (0.45 %, 0.30 % and 0.15 %). Furthermore, the diets were supplemented with bacterial Phytase. Birds were fed ad libitum and kept under thermo neutral conditions. The parameters studied were; body weight gain (BWG), tibial bone characteristics (TBC), serum Calcium (Ca), Phosphorus (P) and Alkaline Phosphatase (AP) levels and tibia ash percentage (TAP). BWG of the broilers was calculated at weekly interval and remaining parameters were calculated after slaughtering the birds at 35th day. Results suggested that Phytase supplementation at 0.30% NPP (Non Phytate Phosphorus + Bacterial Phytase) increased ($P<0.05$) the BWG, bone length, bone weight, tibiotarsal index, medullary canal diameter and diaphysis diameter however, rubosticity index was reduced to minimum ($P<0.05$) at this dietary level of phosphorous when compared with other groups. Maximum ($P<0.05$) rubosticity index was observed in control group with 0% Phytase. Furthermore, Phytase addition at 0.30 % NPP also improved ($P<0.05$) Ca, P and AP levels in the blood. Phytase supplementation at lower phosphorus level (0.30%NPP) improved BWG and TBC including bone density and bone quality in broilers hence it can be concluded that addition of Phytase at 0.30% NPP may prove beneficial for bone and overall performance in broilers

Keywords: tibia, phytase, diaphysis diameter, rubosticity index

S1- 0436 Impact of Schizochytrium QL- B151 supplemented to diet of CAU No.3 layer on DHA content, egg quality and performance of laying hens

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To evaluate the Schizochytrium QL-B151 powder supplement to the diet of CAU (China Agricultural University) No.3 layer on DHA functional egg production. 0%, 1%, 2%, 3% QL-B151 was added to the diet of CAU No. 3 layer. At 7, 14, 21 and 28 days of feeding experiment, the eggs were processed through ether/petroleum extraction and methyl processing, followed by Gas Chromatography- Mass Spectrometry for detection of DHA, ARA, EPA and other fatty acids. Meanwhile, laying hens' production performance were also evaluated. DHA content significantly increased along with the additive ratio. The DHA content reached 180mg, 230mg and 320mg per egg in 1%, 2%, 3% additive group at 7 days post feeding. The content of ARA decreased slightly in additive group and other fatty acids had no significant change among all samples. In terms of egg yolk color, eggshell thickness, relative weight of yolk and hertz unit, additive group had no significant difference compared to control group. As well there are no significant difference in egg quality indexes, egg laying rate, daily feed intake and feed conversion rate. These results suggest that the additive of QL-B151 algal probiotics to diet don't affect production performance of CAU No.3 layer. However, the DHA level in eggs increased significantly after adding algal power. Just one egg contains more than the amount needed DHA for a daily pregnancy support. Compared to fish oil or similar feed additives, QL-B151 algal powder is stable and easier for feed preparation and storage. In addition, EPA content in egg with algal powder additive was relatively low which is suitable for a broader population. CAU No. 3 layer that provide larger yolk, higher DHA conversion efficiency and enrichment in yolk, which is better than the experimental results of the apricot blossom chicken, are naturally suitable for production of DHA functional eggs.

Keywords: CAU No. 3 layer, Schizochytrium(QL-B151), docosahexaenoic acid (DHA), egg quality, production performance

S1-0437 The effect of low protein diets on production performance and uric acid metabolism in laying hens

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The experiments were conducted to explore the effects of essential amino acids in low protein diets on production performance and uric acid metabolism in laying hens. This study is divided into three tests. In experiment 1, one hundred and twenty 28-week-old Hy-Line Brown hens were randomly divided into two groups, control group (CP=16) and low protein group (CP=10). The diet of the low protein group was the diet of the low protein group with supplement with synthetic amino acids. The low protein group and the control group maintain a consistent level of essential amino acids. The experiment lasted for 6 weeks. In experiment 2, twenty 34-week-old Hy-Line Brown hens were randomly divided into two groups, adding Cr₂O₃ in diets. Collect the acquisition of ileal digesta on the fourth day. In experiment 3, twenty 34-week-old Hy-Line Brown hens were randomly divided into two groups. Feeding the 30g diet and then collected blood wing vein at 2, 4 and 6 hours. The production performance of low protein group was significantly lower than the control group ($P < 0.0001$), and head neck feathers were shedding ($P < 0.0001$). Xanthine oxidase and glutamine synthetase in liver homogenate were significantly different ($P < 0.05$). The low protein group of aspartic acid and glycine digestion rate was significantly higher than that of the control group ($P < 0.05$). The digestion rate of alanine higher than the control group. Low protein group of plasma total free amino acid (TAA) level was significantly higher than the control group in 2 and 4 hours ($P < 0.05$). Valine, isoleucine, threonine, methionine, lysine, tryptophan, glutamate and ornithine levels of the two groups have significant differences in amino acid composition ($P < 0.05$). Supplementary crystal amino acids of low protein diets can significantly reduce the production performance of laying hens. This result may be related to amino acid release rate and digestibility of corn soybean meal diet related.

Keywords: low protein, amino acid, release rate of amino acid, digestibility of uric acid

S1-0438 Effect of graded levels of cold pressed canola meal on egg production parameters in brown laying hens

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Canola meal (CM) has been available for layer feeds in Australia for over 30 years. However “fishy taint” caused by trimethylamine (TMA) in egg yolk has limited its inclusion. Genetic changes have improved the defective, recessive FMO3 gene in brown shelled layers resulting in elimination of the problem. The industry however is still reluctant to use high levels of CM. This study was designed to determine the optimum inclusion rate of CM in brown layer diets without compromising production. Cold pressed canola meal was used, containing 110 g/kg oil, 11.09 MJ/kg AME, 350 g/kg CP and 16.2 g/kg SID lysine. Ninety nine Hy-Line brown laying pullets (aged 21 weeks at 50% production) in single cages were employed with three dietary levels (0, 100 or 200 g/kg) of CM. Wheat-based diets were formulated to Hy-Line brown standards for 21 to 41 weeks of age. There were 33 replicates in each group. Initial and final body weight, egg number and egg weight (daily), feed intake and FCR (monthly) and egg quality (shell breaking strength, shell deformation, shell reflectivity, albumen height, yolk colour and shell thickness) were measured. Egg quality was measured at the beginning and end of study. Data were analysed with SPSS version 22. There was no significant difference ($P > 0.05$) found on feed intake (118.9, 117.6, 115.7 g/b/d), FCR (1.94, 1.93, 1.94 g feed/g egg), hen day egg production (97.7, 96.5, 96.6%), egg size (63.0, 62.9, 61.5 g) and daily egg mass (61.5, 60.7, 59.5 g/b/d) for the whole production period for control, 100 and 200 g/kg treatments. Similarly no changes ($P > 0.05$) were observed between treatments on egg quality including shell breaking strength, shell reflectivity, shell deformations, albumen heights, Haugh unit, yolk colour, shell weight and shell thickness. No mortality was observed during the 20 week study. It is concluded that the cold pressed CM can be added up to 200 g/kg in brown laying hen diets without compromising production performance or egg quality.

Keywords: Hy-Line brown, canola meal, inclusion level, egg production, egg quality

S1-0439 Effects of NSP enzymes and prebiotic on growth performance and digestive physiology of broilers

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A 5-wk feeding experiment with 800 one-day - old Ross 308 of broiler chickens was conducted to study the efficacy of NSP enzymes and prebiotics mannanoligosaccharides (MOS) on digestive processes. The birds were fed by the same diet from 1 to 35 day ad libitum with a crumbled wheat - soybean meal based diet (C group) supplemented with 15% triticales (T group); 15% triticales and NSP enzymes (E group, NSP enzymes dosage 0.05 g/kg); 15% triticales and NSP enzymes and prebiotics (EP group, NSP enzymes dosage 0.05 g/kg plus MOS dosage 2 g/kg). At the end of each period (1-7, 8-21 and 22-35 days), body weight (BW), and feed conversion ratio (FCR) were measured. At the end of experiment (day 35), five bird from each group was sacrificed for histomorphometry duodenum and ileum villus lengths and crypt depth which were examined using an "Olympus BX63" microscope, "Olympus DP72" video camera and system (Olympus) of a computer program "Image Pro Plus". The concentration of short-chain fatty acids (SCFA) in the remaining Cecum content were determined by HPLC system (Varian Inc., USA). During the total feeding periods there were no significant differences on BW and FCR between treatments. Supplementation with NSP enzymes and MOS increased the length of ileum villi by 119.62 μm , the depth of crypts- 19.99 μm ($P < 0.05$). In the T group the depth of duodenum and ileum crypts decreased by 29.17 μm and 19.61 μm respectively ($P < 0.05$). Also there were determined, that in the same group the propionic concentration was by 0.34 $\mu\text{mol/g}$ lower in comparison to the C group. The diet supplementation with enzymes and MOS lowered the quantity of butyric acid by 9.73 $\mu\text{mol/g}$ ($P < 0.05$). In conclusion, NSP enzymes and prebiotic can improve gut health, feed digestion and absorption through the positively changed histomorphometry of duodenum and ileum.

Keywords: broilers, digestive physiology, enzymes, prebiotic, SCFA

S1-0440 Effects of complex probiotics on growth performance and carcass traits of Chinese yellow broilers fed wheat-soybean diet

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This study assessed the effects of complex probiotics on performance and carcass traits of Chinese yellow broilers fed a wheat-soybean based diet from day 1 to day 42 of age. The complex probiotic consisted of *Bacillus subtilis*, *Saccharomyces cerevisiae* and *Aspergillus oryzae*. A total of 540 birds were allotted to 3 treatments, each of which consisted of 6 replicate pens with 30 birds per pen. Birds fed a diet based on corn-soybean meal served as positive controls (C). All other birds were fed wheat-soybean meal diets with 100 kcal/kg less metabolic energy, iso-nitrogenous and supplemented with enzymes (xylanase, β -dextranase, cellulase and amylase; wherein one group was the wheat-type control (W) and the other received supplemental complex probiotics (P). Average daily gain (ADG) of P birds increased by 4.15% compared to W ($P < 0.05$), and was not significantly different from C ($P > 0.05$). The feed/gain (F/G) ratio of P was 2.65% less than W ($P = 0.07$), also not significantly different from C ($P > 0.05$). Carcass traits: the eviscerated percentage of P was 1.12% ($P = 0.06$) and 1.35% ($P < 0.05$) greater than that of W and C respectively; similarly, semi-eviscerated percentage of P was 2.19% ($P = 0.08$) and 2.35% ($P = 0.06$) greater than that of W and C respectively; the breast muscle percentage of P increased by 8.57% compared to that in the W ($P < 0.05$), and was not significantly different to C ($P > 0.05$). Interestingly, the birds fed wheat diet had lower shear force and meat yellowness values (b^*) of breast muscle compared to that birds fed the corn diet ($P < 0.05$). It is concluded that complex probiotics improved the performance and carcass traits of birds fed wheat-based diets. For broilers, corn can be totally replaced with wheat plus complex probiotic, saving energy and improving meat quality.

Keywords: complex probiotic, wheat-soybean diet, Chinese yellow broilers, growth performance, carcass traits

S1-0441 Comparing two techniques for viscosity measurements in poultry feedstuffs: does it render similar conclusions?

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Viscosity of intestinal contents is known to affect digestion and absorption of nutrients in poultry. In most poultry studies intestinal viscosity has been measured only after complete removal of solid particles by centrifugation. Centrifugation may however remove particles that contribute to viscosity, hence giving rise to an underestimation of digesta viscosity. Two viscosity measurement techniques, one including a centrifugation step and the other without, were compared in an in-vitro model to investigate whether both techniques result in similar conclusions regarding viscosity. Two sets of feedstuff preparations were used. The first set was prepared with different combinations of milled feedstuffs: 100% corn, 25% corn + 75% wheat, 100% wheat, 90% wheat + 10% rye, all mixed with distilled water in order to have a wide range of viscosity. In the second set, barley was incubated with different beta-glucanases, and soybean and sunflower meal were incubated with different pectinases, all mixed with distilled water. Viscosity was assessed using both techniques (with and without centrifugation) at 6 time points. To evaluate the extent of agreement between the two methods, the Lin's Concordance Correlation Coefficient (CCC) was assessed using the percentage of increase in viscosity (within each method), based on pairwise feedstuffs comparison (first set), or relative to the feedstuff without enzyme (second set). The rate of the agreement between the two methods, was substantial for the first set of feedstuffs (66%) and for the barley diets incubated with beta-glucanases (69%), whereas the CCC score for the soybean meal diets was very poor (2%) and fair for the sunflower meal diets, incubated with pectinases (32%). The latter can be explained by the lack of viscosity in these mixtures anyhow. Although the two techniques are considerably different (e.g. with or without preceding particle removal), they seem to render similar conclusions when applied to poultry feedstuffs.

Keywords: poultry feedstuffs, viscosity measurement, centrifugation, Concordance Correlation Coefficient

S1-0442 Assessment of different nutritional densities for the slow-growing line of female Chinese yellow broilers between days 85 and 120

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This study investigated the effects of different nutritional densities on performance, carcass traits and meat quality of a slow-growing line of female Chinese yellow broilers from day 85 to day 120 of age. A total of 720 birds were allotted to 4 treatments which consisted of diets with low (L), normal (N) and high (H) nutritional densities along with another (S) meeting the Chinese feeding standard for chickens (2004). The N diet, aimed at satisfying the nutrient requirements of the slow-growing line of Chinese yellow broilers, was developed in-house. The metabolic energy of L and H diets decreased or increased by 5% from N and crude protein and all other nutrients such as lysine, methionine, calcium, phosphorus, vitamins and minerals were decreased or increased by 10%. Each treatment contained 6 replicate pens with 30 birds per pen. There were significant differences among the four diets for ADFI, ADG and F/G ratio ($P < 0.05$). ADFI of the birds fed the N diet was greater than for the H diet. Broilers fed the N diet had the best ADG, and lowest F/G ratio and cost of gain ($P < 0.05$). Carcass traits: the eviscerated percentage of the N diet was greater than both H and S diets ($P < 0.05$); the breast muscle percentage in the S diet exceeded that of the H diet ($P < 0.05$), but did not differ from that of N ($P > 0.05$). Meat quality: broilers fed the N diet had the highest pH value, the least drip loss and the least shear force of breast muscle ($P < 0.05$). Therefore, on the basis of the above traits, the N diet, specifically developed for the slow-growing line of female Chinese yellow broilers between days 85 and 120, was optimal.

Keywords: different nutritional densities, slow-growing Chinese yellow broilers, performance, carcass traits, meat quality

S1-0443 Butyrate of different origin affects intestinal drug- metabolizing cytochrome P450 enzymes in chicken

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Butyrate, widely used as an additive in poultry nutrition, can modify the expression of certain genes, such as those of drug-metabolizing cytochrome P450 (CYP) enzymes. Since intestinal CYPs provide a primary metabolic barrier against orally ingested xenobiotics, their dietary modulation can be of high relevance. In the present study, Ross 308 broilers (n=22/group) were fed with maize-based or wheat-based diet, containing lower or higher non-starch polysaccharide (NSP) levels, the latter stimulating caecal bacterial butyrate production. Diets were supplemented with non-protected (1.5 or 3.0 g/kg diet) or protected butyrate (0.2 g/kg diet). The kinetics of butyrate was assessed from ingesta and portal blood by gas chromatography; CYP activities were measured from duodenal mucosa with luminescent assays. Data were analyzed by two-way ANOVA and pairwise comparison using the R 2.14.0 software. Ileal butyrate concentration was increased by protected butyrate, while NSP-rich diet resulted in elevated caecal butyrate levels. According to differing absorption sites, butyrate of different origin increased butyrate levels in various vessels of the hepatic portal system. Duodenal CYP1 and CYP2 activities were increased by non-protected butyrate supplementation and by wheat-based diet, while CYP3 was affected by the type of cereal only. This stimulatory action of butyrate of different origin on intestinal CYPs can be of high importance from food safety and pharmacological point of view by possibly modifying the metabolism of simultaneously applied drugs. This study was supported by CEPO (Centre of Excellence for Poultry) project, funded by the European Regional Development Fund, Cross-border Cooperation Programme Austria-Hungary 2007-2013.

Keywords: feed additives, butyrate, drug metabolism, xenobiotics

S1- 0444 Determination of optimal reduction of dietary Ca levels in the finisher phase of broiler feeds

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This study aimed to determine the maximum reduction in dietary Ca that can be applied in broiler finisher diets to maximise performance without impairing bone quality. Lowering dietary Ca is known to enhance the efficiency of phytase thereby improving the absorption of P naturally present in feedstuffs in the form of phytate. The study followed a complete block design experiment, in which Ross 308 day old chicks (42 birds/pen) were allocated on each of the 124 pens. At placement, birds received the same starter feed (0-8d) containing 9 g Ca/kg. Subsequently, during the grower phase (8-24d), some birds received the Control treatment (7.5 g Ca/kg) or low Ca diet (5.5 g/kg) with 500 or 1000FTU of phytase. Those birds on the Control received a relative high Ca diet (6.2 g/kg) in the finisher phase (24-42d), whereas those on the low Ca diet received several Ca levels (3.5, 4.0, 4.5, 5.0 g/kg) with either 500 or 1000FTU of phytase. Diets were formulated at the same dP for each feeding phase (4.6, 3.5 and 2.9 g/kg for starter, grower and finisher, respectively). Animal performance, bone mineralisation and incidence of broken bones at slaughterhouse were evaluated. Lowering dietary Ca did not affect BW, feed intake and FCR compared to Control neither for the entire global period (0-42d) nor for the finisher period (24-42d). Although tibia ash contents (%DM) were not statistically affected by dietary treatment, tibia breaking strength was smaller ($P < 0.05$) than the Control treatment when 3.5 or 4.0 g Ca/kg and 500FTU were used. In contrast, bone quality parameters did not differ from the Control when 4.5 g Ca/kg or greater Ca levels were used regardless the phytase level. Analysis at the abattoir only detected a greater incidence of broken bones relative to Control with the 3.5 g Ca/kg and 500FTU. The outcomes of this study suggest that 4.5 g Ca/kg in the finisher phase could be sufficiently safe to ensure an adequate bone mineralisation without impairing animal performance.

Keywords: calcium, phosphorous, broilers, finisher, bone mineralisation

S1-0445 Effect of dietary corn distillers dried grains with solubles on growth performance, antioxidation, intestinal immunity and meat quality of yellow-feathered broilers

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The experiment investigated the effects of different dietary levels of corn DDGS on growth performance, antioxidation, intestinal immunity and meat quality in Lingnan yellow-feathered broilers aged from 1 to 63 d. 1440 Lingnan male hatchlings were randomly assigned to 6 treatments, each consisting of 6 replicates of 40 birds. The basal diet (I) was a typical corn-soybean ration while the experimental diets (II to VI) substituted corn DDGS for soybean meal. The level of substitution in starter (d 1-21), grower (d 22-42) and finisher (d 43-63) diets was: II, 2%, 4% then 6%; III, 4%, 8% then 12%; IV, 6%, 12% then 18%; V, 8%, 16% then 24%; VI, 10%, 20% then 30%. A sample of birds from each replicate was blood sampled and killed at the end (d 63) for carcass and biochemical analysis. Compared to the control diet (I), diets II to VI increased ADG and F:G between d 1 to 21 ($P < 0.05$), but there was no differences from diet in BW at d 63 and ADFI, ADG, F:G between d 1 to 63 ($P > 0.05$). Also there was no differences from diet in plasma GSH, T-AOC and activities of T-SOD, GSH-Px ($P > 0.05$), but with the increasing of corn DDGS, the plasma content of MDA showed an increasing trend ($P = 0.07$). The jejunum IL-6 relative gene expression and sIgA content in diet V to VI was significantly lower than that in the control group ($P < 0.05$). In addition, increased corn DDGS content of the diet caused favorable changes in selected functional properties of the meat, diet V to VI increased drip loss at 24h and 96h in breast meat ($P < 0.05$). Further, diet VI decrease in L^* value and increase in a^* value in broilers ($P < 0.05$). No differences existed among breast meat from the different treatments with respect to shear force, pH and b^* values at 45 min and 96h after slaughter ($P > 0.05$). In conclusion, Diet VI (10%, 20% then 30% corn DDGS) in Lingnan yellow-feathered broiler diets have no negative effects on growth performance, antioxidant capacity, but higher inclusion levels can lower meat quality.

Keywords: corn distillers dried grains, yellow-feathered broiler, growth performance, antioxidation, intestinal immunity, meat quality

S1-0446 The effects of dietary vitamin E and selenium supplementation on reproductive performance, egg quality of Chinese yellow-feathered broiler breeders and hatch weight of chicks

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The effects of dietary vitamin E (VE) and selenium (Se) supplementation levels on reproductive performance, antioxidants levels of Chinese yellow-feathered broiler breeders and hatch weight of chicks were studied. A total of 864 birds were blocked on the basis of body weight, and then randomly assigned to 1 of 9 treatments in a 3×3 factorial arrangement with 3 levels of VE (0, 20, 40 IU/kg) and Se (0, 0.15, 0.30 mg/kg) for a 8 wk study period. Each dietary treatment had 6 replicates with 16 birds per replicate. At 4, 8 wk of treatment, 4 eggs per replicate were collected for detecting egg quality. After 8 wk of treatment, 50 eggs per replicate were collected for hatching. There were no significant differences in egg laying rate, average egg weight and feed efficiency among all treatments ($P > 0.05$). Egg quality, at 4 wk of treatment, the egg-shell thickness of (40×0) group was significantly higher than (20×0.15) group ($P < 0.05$); the egg-shell weight of (20×0) group was significantly greater than (40×0.30) group ($P < 0.05$). There was no significant difference in egg quality among all treatments at 8 wk of treatment ($P > 0.05$). The number of follicles (40×0.15) group was significantly more than (0×0) group ($P < 0.05$). There were no significant differences in the weight of offspring, hatchability, fertilization rate and the rate of embryonic death among all treatments ($P > 0.05$). Therefore, according to the results of egg laying performance, egg quality and the weight of offspring, it is implicated that normal corn-soybean meal diet (not feed materials of Se deficiency area) contains enough Se to support production of Chinese yellow-feathered broiler breeders, VE maybe improve the performance of Chinese yellow-feathered broiler breeders.

Keywords: VE, Se, Chinese yellow-feathered broiler breeder, hatch weight of chicks

S1-0447 Regulation of ghrelin on lipid metabolism of poultry

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Ghrelin is a metabolic hormone that promotes energy conservation by regulating appetite and energy expenditure. Although many researches on the physiological role of ghrelin regulating energy metabolism, there is little information about the metabolic actions of ghrelin in poultry. In this study, we investigated gene expression of ghrelin in response to energy status of chickens, to find out the mechanism of ghrelin regulation of lipid metabolism to maintain energy balance. Acylated ghrelin (1nmol) to deal with poultry hepatocytes, myoblasts and adipocytes, TG, lipid metabolism related genes were detected and related signaling pathways. Results: (1) We compared ghrelin mRNA expression in different animal species (Kunming mice, pigs, Hyline layers, and broilers). The glandular stomach is the main organ of ghrelin expression in poultry, followed by duodenum and abdominal fat. GHSR-1a mRNA is expressed in the glandular stomach, duodenum, liver and abdominal fat. (2) Blood glucose concentration was decreased, meanwhile the concentration of plasma ghrelin was increased significantly by fasting, high-energy diet and insulin treatments. Ghrelin expression in gastric gland and hypothalamus in fasting and high-energy diet groups were significantly increased. GHSR-1a expression of abdominal fat in fasting group and of hypothalamus in high-energy diet group were significantly increased. Ghrelin and GHSR-1a expression did not change by insulin treatment. (3) Treatment of cells with ghrelin significantly decreased TG deposition, increased lipolytic enzyme activity, and upregulated AMPK protein levels. Conclusion: The present result suggests that ghrelin could promote fat oxidation to maintain the body's energy balance, through AMPK signaling pathway.

Keywords: ghrelin, GHSR-1a, lipid metabolism, AMPK

S1-0448 Heat production estimated from fasting layer hens at peak lay

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Fasting heat production in animals are a good indication of energy required for basal metabolism for maintenance. The estimation of maintenance energy is necessary for determination of heat increment of animals due to metabolism and activities, which is important components in the assays to measure the net energy of diets and feed ingredients. In poultry, fasting heat production (FHP) has been well established in general. However, that of layer hens has not been extensively investigated. In the present study, an open circuit respiratory calorimetric chamber system was employed to measure the FHP and respiratory quotient (RQ) of layer hens according to the Brouwer equation. Eighteen birds at 27 wk of age with the mean body weight of 1.91 kg were allocated to 6 calorimetric chambers. The birds were housed under dark and optimal temperature and humidity. The birds were fasted for 9 hours and measurements were then performed for another 24 hr with fasting. The results demonstrated that oxygen consumption and CO₂ exhale were highest during morning and lowest during night. These measurements attained lowest at 17:30 and kept stable for approximately 11 hr until 4:00 am. The RQ and FHP at zero activity was 0.680 and 380 kJ per day per kg of metabolic body weight respectively. Those were 0.716 and 464 kJ per day per kg of metabolic body weight respectively while heat produced from activity during the fasting were included. The data indicated that layer hens produce less heat for maintenance compared with broiler chickens.

Keywords: fasting heat production, respiratory quotient, layer

S1-0449 Effect of benzoic acid protection of broiler performance and bedding physicochemical properties

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The effects of dietary benzoic acid supplementation on performance and bedding were investigated in broiler chickens. 2400 1-day-old male broiler chickens were randomly allocated to 24 pens of 100 birds. The chickens from 12 pens were fed with basal diet while the others were fed with basal diet supplemented with benzoic acid (500g/T). Results showed that (1) dietary supplementation of benzoic acid had no effect on average weight gain, average feed intake, FCR and mortality rate. (2) Dietary supplementation of benzoic acid reduced the occurrence of foot pad dermatitis and hock burns. (3) Dietary supplementation of benzoic acid had no effect on humidity of bedding. (4) Dietary supplementation of benzoic acid significantly decreased the pH of bedding. (5) Dietary supplementation of benzoic acid reduced the TN content of bedding. The study showed that dietary supplementation of benzoic acid has no effect on performance, but it considerably changes the physicochemical property of bedding.

Keywords: average weight gain, average feed intake, feed conversion, mortality, foot pad dermatitis, hock burns, bedding moisture, pH, total nitrogen content

S1-0450 Supplementation of L-threonine for optimization of dietary crude protein levels in commercial broiler chickens

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A study was conducted with the objective of optimizing the dietary crude protein levels for growth and carcass characteristics in commercial broiler chickens with supplementation of L-threonine. Four hundred and sixty two day-old Vencob broiler chicks were randomly allotted to seven treatments with six replicates each and eleven chicks per replicate according to a completely randomized design. Crude protein levels and L-threonine supplementation were as per the NRC recommendations of 1994. T1 was control. Step down crude protein reduction was followed by 0.75, 1.50 and 2.25 per cent without L-threonine in T2, T4 and T6 and with L-threonine supplementation for T3, T5 and T7, respectively. The feed was offered in mash form during pre-starter (0-14 days), starter (15-28 days) and finisher (29-42 days). The data were subjected to statistical analysis by one way analysis of variance with Tukey's multiple comparison test using Graph-Pad prism 5.01 statistical software at 95% level of significance. At 42 days, the body weight gain and feed intake were reduced significantly by step down reduction of crude protein levels without L-threonine supplementation in T2, T4 and T6. Whereas, body weight gain and feed intake were not affected at 0.75 and 1.50 per cent reduction of crude protein with L-threonine supplementation compared to control, but not at 2.25 per cent. The feed to gain ratio at crude protein reduction of 0.75 per cent with L-threonine supplementation was comparable with the control group. The carcass characteristics such as dressing percentage, breast yield, thigh muscle yield, drumstick yield and abdominal fat percentage at crude protein reduction of 0.75 and 1.50 per cent remained constant compared to control. In conclusion, since the growth performance and carcass characteristics were not affected with L-threonine supplementation at 1.50 per cent crude protein reduction, it could be used as an optimum crude protein level for commercial broiler chickens.

Keywords: broilers, threonine, crude protein, carcass characteristics

S1-0451 Improving fat and gross energy digestibility of broilers by adding an emulsifier to fat powder containing diets

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The effect of a dietary emulsifier (0.01%), with a hydrophilic-lipophilic balance (HLB) of about 17-18, was evaluated on the digestibility efficiency of male broilers (Ross 308). The digestibility coefficients (DC) were determined using the total faecal collection method at two ages (11-15d and 18-22d). A 2x2x2 factorial design was used, with two diets, with and without emulsifier, and at two ages. Diet A had a high free fatty acid (FFA) content (20.0%) and an unsaturated/saturated FA ratio (U/S) of 1.60; while diet B had a FFA of 10.0% and an U/S of 2.00. Both diets had the same calculated ME (12.27MJ/kg) and crude fat (8.5%) content. As lipid sources, soy oil and two types of fat powder were used in both diets; and also animal fat in diet B. The emulsifier was added on top, resulting in four dietary treatments. There were no significant interactions (two- or three-factorial) between age, diet and addition of emulsifier. When diet A (with and without emulsifier) was fed, the crude fat (CF) and gross energy (GE) DCs were significantly ($P < 0.001$) lower compared to diet B (67.3% vs 74.1%, and 71.9% vs 74.0%, resp.). The addition of the emulsifier significantly ($P < 0.01$) improved the CF and GE digestibility (72.6% vs 68.9%, and 73.5% vs 72.4%, resp.), and significantly ($P < 0.01$) enhanced the energy efficiency (ME_{en}) with 220 kJ (1.6%). It can be concluded that, when using an emulsifier, diets with different fat compositions and even containing fat powder, can be formulated for a lower energy content and by consequence reduced feed cost.

Keywords: broiler, emulsifier, digestibility, fat, energy

S1-0453 Effect of dietary supplementation of *Bacillus subtilis* on the growth performance and intestinal microarchitecture in broiler chickens: a comparison to antibiotic effect

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The current study is designed to compare the effects of probiotic with antibiotic on growth performance (weekly basis) and histomorphological modulations in the small intestinal mucosa (Villus height, VH; Villus width, VW; Villus surface area, VSA; Crypts depth, CD; Villus height and crypts depth ratio, VH:CD) in broilers. A total of 120, 1-d-old chicks were randomly divided into 4 treatments (30 per group) with 3 replicates in each group. Subject to the addition per kg diet, 4 groups consisted of, one control (C, without any addition), one antibiotic (A, containing 10% Zinc bacitracin), or (PL0.5 and PH 0.1, *B. subtilis*-containing probiotics at two inclusion levels (0.05 and 0.1 g/Kg of feed, respectively) for 35 days. Water and feed were provided ad-libitum. The birds were weighed weekly to determine weight gain and feed conversion ratio. Two birds from each replicate were slaughtered on d-21 and d-35 and tissue samples from small intestines were processed for microarchitecture. Results showed that the broilers fed with probiotic had numerically more mean body weight and weekly weight gain compared to the non-supplemented or antibiotics-fed group during starter phase. In the grower phase the probiotics along with group A reflected superior ($P < 0.05$) performance compared to group C. Throughout the experiment, PH- 0.1 group had almost similar FCR compared to PH-0.5 and A, and lower ($P < 0.05$) compared to that of group C. On d-21, in duodenum and jejunum and on d-35 only in ileum the VH, VSA, VH:CD were improved ($P < 0.05$) in both probiotic groups compared to groups A and C. The VH and VSA in ileum increased only in PH-0.1 ($P < 0.05$) on d-21 compared to groups A and C. On d-35 the VH in duodenum and ileum (as well as aforementioned) improved ($P < 0.05$) in both probiotics groups compared to A and C. In conclusion, dietary supplementation of *Bacillus subtilis* at 0.1g/kg of feed may improve the growth performance in broiler chickens.

Keywords: broiler chicken, *Bacillus subtilis*, growth, intestinal histology, villi

S1- 0454 The advantage of donor polynuclear matrices of essential trace elements in appropriate providing of chicken nutrition needs

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Nowadays, there are many decisions concerning the use of chelate and coordination compounds of trace elements. As a rule, it's economically inefficient to substitute of non-organic premixes completely. Therefore, these coordination compounds are considered as an instrument for regulation of biochemical and physiological processes in bird's organism. The main aim of this study was to research the efficiency of a new polynuclear coordination compound. Chickens were kept in battery cages in compliance with the recommended process parameters. Experimental group was received feed with increased levels of acid and peroxide number and new additive. Blood samples were collected at 3, 7, and 14 days after the introduction of additives and after their withdrawal. At the end of the experiment the slaughtering was performed (5 heads per group). The choice of bimetals as a partner substance was explained; methods for the synthesis of new compounds were suggested with main cluster complex which includes complexes of six trace elements (Cu, Fe, Zn, Mn, Co, Cr) with N-(2,3-dimethylphenyl)anthranilic acid with molar ratio 50:50:15:10:1:1. Presence of synthesized compound was confirmed by mass spectrometry, x-ray diffraction and nuclear magnetic resonance. Its composition and structure were also determined. The new compound is a polynuclear cluster, a complex with a carboxylated bridge type of chemical bond (Chinese lantern structure). It was shown that biological effect of compound takes place with concentration from 3, 0 to 20,0 mg/kg of broiler's weight, and with 250 mg per 5 g (feedstuff per volume of intestinal microbiota). The use of this polynuclear compound with marginal lipid constants (peroxide, acid and TBA), as was showed in experimental studies on hens and broilers, reduces the process of lipid peroxidation (concentration of MDA in blood serum and tissues) and increases activity of antioxidant enzymes ($p \leq 0,05$), as well as productive traits.

Keywords: polynuclear coordination complexes, chickens, microbiota

S1-0455 Effects of different tea polyphenols products on laying performance, Serum lipid level and egg quality in laying hens

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The experiment was conducted to study the effects of different purity and levels of tea polyphenols products on laying performance, serum lipid level, and egg quality in laying hens. A total of 1200 Lohmann layers (65-week-old) were randomly assigned to 1 of 10 diets involving 1 control plus 3×3 factorial completely randomized arrangement. The 3 tea polyphenols products were the 3 tea polyphenols content of 20% (TP20), 30% (TP30), 60% (TP60) with 3 tea polyphenol supplementation levels at 0.066% , 0.133% , 0.266%. Each treatment consisted of 8 replicates of 15 hens each. The duration of the trial was 9 weeks. The results showed that: (1) No significant difference was observed on performance by tea polyphenols products, interaction of products and level ($P>0.05$). At the experiment 1- 9 weeks, TP30 and TP 60 decreased the ADFI significantly ($P<0.05$). The addition of TP30 at 0.266% had the lowest egg production, egg mass, egg weight, ADFI, with the highest mortality, and feed to egg ratio ($P<0.05$). Addition of 0.266% TP significantly decreased the laying performance, egg mass, ADFI and feed to egg ratio ($P<0.05$). There was no significant difference in laying performance, egg mass, ADFI, and feed to egg ratio when fed the diets with TP supplemented level at 0.066% ($P>0.05$). (2) The addition of TP20, TP30, TP60 decreased the serum total cholesterol (TCH), triglyceride (TG), LDL-C, and increased the HDL-C significantly at 6 weeks ($P<0.05$), but no significant difference at 9 weeks. (3) There was no significant influence on egg shell quality by TP products, interaction of products and levels ($P>0.05$). TP30 or TP60 increased the egg Hough unit significantly ($P<0.05$). The addition of TP30 had the highest cracked and broken, soft, with the lowest egg-shell thickness ($P<0.05$). 0.266% TP significantly increased the soft egg percentage ($P<0.05$). In conclusion, The suitable supplemented TP level in corn-soybean meal diet was 0.133% TP60.

Keywords: tea polyphenols, performances, lipid metabolism, egg quality

S1-0457 Beta-alanine as a factor influencing the content of bioactive dipeptides in muscles of broiler chickens and Ayam Cemani chickens

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Carnosine (L-Car) and anserine (L-Ans) are endogenous dipeptides found in skeletal muscles. The content of these peptides increases along with a decreasing content of chromoproteins, e. g. hemoglobin, myoglobin. We hypothesized that the supplementation with beta-alanine can increase the content of carnosine and anserine in muscles of chicken. The aim of this study was to determine the effect of various doses of beta-alanine administered in feed mixtures and of heat treatment on changes in the contents of dipeptides: anserine and carnosine, in meat of broiler chickens and Ayam Cemani. This experiment was conducted using broiler chickens and Ayam Cemani aged 42 d. The differentiating factor was the content of powdered beta-alanine (Olimp Sport Nutrition Beta-Alanine Xplode® Power) supplemented in basal feed mixtures, i.e. the control group (Control) did not receive beta-alanine supplementation whereas group (B1broiler and B2broiler) (A1cemani and A2cemani) received was receiving 0.1% and 0.5% addition of beta-alanine in powdered form, respectively. Carnosine and anserine were extracted from chicken meat and analyzed by high performance liquid chromatography (HPLC) using methods adapted by Mora et al. 2008. The study demonstrated that interesting effects might occur during beta-alanine administration to feed mixtures for chickens, especially including increased contents of anserine and carnosine in skeletal muscles of the birds fast growing.

Keywords: anserine, carnosine, muscle, poultry, Ayam Cemani

S1-0459 Effects of high-oleic vegetable oils and level of inclusion on growth of newly hatched chicks

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Oleic acid (OA) is the main fatty acid present in the triacylglycerol lipid of the yolk sac, the major energy source for the growing chicken embryo. As OA is highly absorbed close to and after hatch this study investigated whether vegetable oils with high OA content could be an energy source for newly hatched chicks. The experimental design consisted of a 3 x 2 factorial design to study the effect Low-OA (20.8% OA), High-OA1 (74% OA) or High-OA2 (85.4% OA) oils at 3 or 6% level of inclusion. Each treatment was repeated 12 times and each experimental unit was composed of 56 male Ross 308 chicks. Pre-starter diets based on corn-wheat-soy bean meal were isocaloric (2850 Kcal AME broiler); oil inclusions were added at the expense of main energy-yielding ingredients. Day old chicks received experimental diets from placement to 4 days of age after which all received a common standard starter, grower and finisher diets. Yolk sac absorption at 3 days was measured as well as performance parameters for the experimental period (0 - 4 days) and global grow-out period (0- 36 days). Statistical analyses were performed using Proc Mixed model at $P < 0.05$ significance level. Dietary oil source did not influence chick's body weight at 4 days of age; yet chicks fed High-OA2 diet consumed more feed relative to those fed Low-OA. Chicks fed High-OA1 were intermediate to and not significantly different from the other feeding treatments. Regardless of oil source, chicks fed diet with 6% oil were about 2.5% lighter in comparison to those fed 3%. This effect remained until 28 days of age and was no longer observed at market age. Increased oil level decreased feed efficiency of newly hatched chicks and this effect was more evident when diets were supplemented with 6% Low-OA. Residual egg yolk absorption was not influenced by experimental diets. Despite no influence of oils rich in oleic acid on development the increased dietary fat in pre-starter diets reduced growth of newly hatched chicks.

Keywords: oleic acid, pre-starter, egg yolk, performance

S1- 0460 Designing arabinoxylan structure by enzymatic degradation to improve the performance and the digestive health of wheat-fed broilers

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Carbohydrate degrading multi-enzyme preparations (MEP) are used as feed additives to improve broiler performance. Their mode of action is complex and not fully comprehended. In this study we investigated the effect of wheat degradation-products obtained after MEP treatment on broiler performance, on the intestinal microbiota, on L-cells and on T-lymphocytes during a 2-weeks in vivo experiment. Water-soluble fractions were isolated at pilot scale from wheat grain with (WE) and without (WC) MEP treatment. Sub-fractions were further obtained from WE and WC by graded ethanol precipitation. Sub-fractions WE-1 and WC-1 were recovered in 65% ethanol and sub-fractions WE-2 and WC-2 in 80% ethanol. The fractions were incorporated at 0.1% in a wheat-based diet to feed 480 Ross PM3 broilers distributed in 5 groups of 96 (12 broilers x 8 pens), one negative control group (NC) and 4 treatments groups. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated. At day 14, all the animals were euthanized and from 24 animals/group ileal and cecal contents and tissue samples were collected. MEP degradation increased the solubility of arabinoxylans (AX) and reduced their Mw and polymerization degree (DP) but no oligosaccharides were produced (DP>10). The AX represented 50% of WE-1 vs. 15% of WE-2, whereas they represented 45% of WC-1 vs. 1% of WC-2. The WE-1 significantly ($P<0.05$) increased BWG by 14% after the first week post hatch and 6% throughout the 2 weeks, increased bacteria of the Lachnospiraceae family, increased short chain fatty acid (SCFA) production in ceca (mainly butyrate and acetate), decreased the T-lymphocytes infiltration in the ceca and ileum and increased the L-cells density in the ileal mucosa, when compared to WC-1. The WE-2 had no significant effect on animal performance nor on intestinal health, when compared to WC-2. In conclusion, MEP degradation products improved broiler performances and digestive health during the first 2-weeks post hatch.

Keywords: enzyme, T-lymphocytes, L-cells, short chain fatty acids, body weight gain

S1-0461 The effect of pre-peak feeding strategies on early laying performance

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This study aimed to assess contrasting nutritional alternatives to support early production in layers through feeding strategies that promote total nutrient intake during the pre-peak phase. Transition from rearing to lay is a period in which pullets undergo many changes (transport, environment, diet...) and the rapid increase in egg production is not always synchronized with a sufficient feed intake. Trial design consisted of 3 Diets (Standard; Dense; Low Energy) x 3 Premixes (Control; Metabolic [Organic minerals and higher levels of Vitamin E, C, D and B]; Gut [Presan; an intestinal health modulator]) during the pre-peak phase (15 to 24 weeks). Each of the 9 dietary treatments were randomly distributed across the experimental units ($n=7$), each of them consisting of 22 collectively caged Isa Brown hens. From 25 to 32 weeks of age, the Standard diet with the Control premix was used in all the previous treatments. Pullets were selected at 15 weeks to be 100 g BW lighter than recommended by the breeding company. Pullets were light stimulated at 15 weeks of age to provoke early production in hens with low BW. Results from 15 to 24 weeks of age, showed that a pre-peak diet based on Low Energy, compared to the Standard and Dense diets, increased CP, Ca and P intake 5% on average ($P<0.05$). Moreover, egg production (89.19 vs. 86.45 and 86.58%; $P<0.05$) and egg mass (51.41 vs. 49.66 and 49.53 g/d; $P<0.05$) of the whole period of production studied was higher with the Low Energy Pre-peak diet. Gut Premix (Presan) tended to improve egg production ($P=0.071$) compared to the other premixes. A positive interaction was observed when a Low Energy diet with Presan was fed, obtaining the highest egg production ($P<0.05$) and egg mass ($P=0.058$). As conclusion, the usage of Low Energy diets improved production in early phases and the effect might be higher with the addition of Presan as gut.

Keywords: lay, transition, egg production, pre-peak, feeding strategies

S1-0462 Performance, egg characteristics and lipid profile of eggs of Japanese quail fed varying dietary crude protein diets

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A study was conducted to determine the performance, egg characteristics and lipid profile of laying Japanese quails fed varying crude protein diets in the humid tropics. A total of 120 female quail birds were used. The birds were randomly assigned to four dietary treatment groups of control (T1), T2, T3 and T4 with protein levels of 16.5%, 18%, 21% and 24% respectively. Each treatment was replicated thrice with 10 birds per replicate. Data were collected on the performance, egg characteristics, lipid profile and cost-benefits. Data collected were analyzed using ANOVA in a completely randomized design. Results showed that birds on treatment 3 (21%CP) diet laid significantly ($P < 0.05$) higher number of eggs, had significantly better feed conversion ratio and higher egg weight value of 10.91g which differed significantly from value of 8.86g recorded for birds on the control. Egg volume (cm³) and shell surface area (cm²) were significantly affected by treatments. The same birds on treatment 3 had significantly higher values than the control birds. The total cholesterol of egg yolk of birds on treatment 1 (104.50mg/dl) was the lowest and differed significantly from the highest value of 110.53mg/dl observed for birds on treatment 2. Proximate composition of the eggs revealed that birds on treatment 4 had significantly ($P < 0.05$) higher value of ether extract (10.58%) as compared to the least value of 9.60% observed for birds on treatment 3. Birds on treatment 3 had least cost per egg of N6.5k and also yielded the highest benefit per egg of N16.02. It is concluded that a 21% crude protein diet for quails is optimum for laying in a humid tropical environment.

Keywords: egg, Japanese quail, lipid

S1-0464 The comparative efficacy of an ionophore, vaccine and oregano oil on coccidial immunity in broilers

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This study evaluated an ionophore, anticoccidial vaccine and oregano oil on broiler performance and development of coccidial immunity in Cobb 500 birds reared to 42 days on reused litter. Following a randomised block design, 675 broiler chickens were split into 3 treatments (9 reps/treatment, 25 birds/rep): 1) Salinomycin (S) (Biocox60, Alpharma, USA), 60g/t; 2) anticoccidial vaccine (V) (Coccivac-B52, Merck, USA); 3) Orego-Stim (OS) (Anpario Plc, UK), 450g/t. On day (d) 21, 5 birds per pen were transferred to cages and challenged orally with a combination of *Eimeria* spp. As a control (C), 45 non-vaccinated, coccidia free birds were also challenged. Six days post-challenge, birds were euthanased and scored for coccidial lesions. Litter samples were taken on d7, 14, 21, 28 and 35 to determine total oocysts per gram ($\times 10^3$ OPG) of faeces. Data were analysed by ANOVA. Statistical significance was declared at $P < 0.05$. Final FCR for S and OS was similar, but both were significantly lower than V (S: 1.64, OS: 1.67, V: 1.87, respectively). OPG was low and numerically similar for S and OS until d35, although OS tended to be higher. On d35, OS OPG increased compared to S (2.92 and 0.22 respectively). On d7, V OPG was similar to S and OS. OPG peaked on d14, where they were significantly higher than S and OS (5.62, 0.00 and 2.92 for V, S and OS respectively) before returning to similar levels as the other treatments on d28. On d35, V demonstrated another increase in OPG which was significantly higher than OS and S (5.62, 0.22 and 2.92, respectively). C demonstrated that the challenge model was successful (mean lesion score 3.04). All treatments had significantly lower lesion score compared to C but did not differ significantly between each other (S: 1.53, OS: 1.46, V: 1.20). In conclusion, Orego-Stim was shown to perform as well or better than the other established methods of coccidial control, showing it could be a useful tool in the management of coccidiosis.

Keywords: broilers, coccidiosis, gut health, oregano essential oil

S1- 0465 Effects of increased diet density through increased dietary fat level on growth performance, organ size, and dietary fat metabolizability of broilers during the first week of life

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The perception of suboptimal fat digestion in young broilers has resulted in carbohydrate based diets being considered the best nutritional support during the first week of life. The current study aimed to determine if increased diet density through increased dietary fat level affected growth performance, organ size and dietary fat metabolizability of broilers during the first week of life. Day old broilers were randomly allocated to a dose response design that comprised 5 levels of dietary fat (3.5, 7.0, 10.5, 14.0, and 17.5%). In total 540 broilers were used, with 108 broilers divided over 6 replicates per treatment. The dietary fat level was increased through soybean oil inclusion while maintaining a constant digestible amino acid to energy ratio. Diets were not kept isocaloric nor isonitrogenous. Broilers were weighed on day 0 and 7 and sampled at day 7 for digestive organ sizes. Feed intake and total excreta production were measured from 0 to 7 days to calculate dietary fat metabolizability. Data were analyzed for linear and quadratic responses. An increased diet density through increased dietary fat level resulted in a linearly lowered BW gain ($\Delta=7.2\%$; $P=0.047$) and feed intake ($\Delta=22.9\%$; $P<0.001$) for 3.5 to 17.5% dietary fat level from 0 to 7 days, whilst gain to feed ratio linearly increased ($\Delta=14.4\%$; $P<0.001$). In addition, increased diet density resulted in a linear decrease of relative liver ($\Delta=24.2\%$; $P<0.001$) and pancreas size ($\Delta=12.9\%$; $P<0.001$). On the contrary, intestinal length and weight linearly increased for the duodenum, jejunum, ileum and caecum ($P<0.05$) with an increase of diet density. Dietary fat metabolizability was not affected by treatment. High density diets decreased BW gain and feed intake, but increased feed efficiency. The latter is the logic consequence of the increased diet density. While dietary fat level affected digestive organ sizes at 7 days of age, it did not affect dietary fat metabolizability.

Keywords: broiler chicken, diet density, dietary fat level, pre-starter

S1-0466 Use of Cu nanoparticles in broiler chickens feeding

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Copper is a key trace mineral involved in a variety of physiological processes and is commonly used as a promoter of growth and health in poultry production. Today copper is added, mainly in the form of Cu salts, to feed premixes for broiler chickens owing to its antibacterial and growth-promoting effects. However, only a small fraction of Cu is digested, while the major part is excreted with faeces causing environmental pollution. We hypothesized that copper nanoparticles could be an alternative to Cu salts. Because of their large surface area, the nanoparticles exhibit a very high bio-activity and can be used in much smaller doses than Cu salts. The objective of the study was to determine the efficacy of Cu nanoparticles supplementation into complete feed mixtures for broiler chickens through the evaluation of chicken performance and slaughter analysis. The experimental material included fast-growing Ross 308 broiler chickens. The birds were kept in individual cages. The control and the experimental groups were housed in the same room to avoid differences induced by environmental effects. The chickens were fed ad libitum with complete feed mixtures according to Poultry Feeding Standards. Experimental feed mixtures were supplemented with various doses of Cu nanoparticles replacing 25, 50, 75 and 100 % of CuSO₄. The body weight, feed and water intake were monitored during the experimental period. After completed the experiment, on the 42nd day of age, the birds were slaughtered, their carcasses were air-cooled at 4 °C for 24 h. Afterwards, dissections were performed. It was found that the replacement of the standard form of copper by its nanoparticles affected the body weight and dressing percentage of chickens at the end of rearing period and may reduce Cu excretion into the environment. The study carried out within BIOSTRATEG 1 (GUTFEED) research project, subsidy no NN.267659/7/NCBR/2015.

Keywords: copper nanoparticles, chickens, performance

S1-0468 Maternal and offspring dietary supplemented with vitamin A on growth and meat quality in broilers

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To evaluate effects of dietary VA levels for maternal and offspring on growth, antioxidant ability and meat quality in broiler offspring. A total of 528 yellow-feathered broiler breeders were fed a corn-soybean meal basal diet supplemented with 0, 5,400, 10,800, or 21,600 IU/kg VA. Each dietary treatment had 6 replicates with 22 birds per replicate. After 7 wk of treatment, 60 settable eggs per replicate were collected for hatching. The offspring were respectively selected and allotted into 8 groups, and half of groups were fed with diet containing 5,000 IU/kg VA and the others were fed without VA. Results showed that adding VA in the offspring diet improved BW at d 21, d 42 and d 63, and ADG, ADFI at every stage from 1 to 63d of age ($P < 0.05$), and reduced F/G in broiler offspring from 22 to 63d of age ($P < 0.05$) and mortality of offspring from 1 to 63d of age ($P < 0.05$). Maternal VA did not significantly affect the growth performance and mortality of offspring at every stage from 1 to 63d of age. There were significant interactions between dietary VA levels for maternal and offspring on ADFI during 1 to 21d ($P < 0.05$). There were no significant effects of dietary VA for maternal and offspring on plasma activity of TSOD and GSH-Px and MDA ($P > 0.05$). The interaction between dietary VA of maternal and offspring on the activity of GSH-Px was detected in the plasma ($P < 0.05$). The pH value of breast muscle 24h postmortem increased with dietary VA supplementation of maternal at the levels of 21,600 IU/kg compared to 0 and 5,400 IU/kg ($P < 0.05$). VA addition in offspring diet increased breast muscle shear force ($P < 0.05$), pH value ($P < 0.05$) 24h postmortem, and decreased meat color L* value ($P < 0.05$) and b* value ($P < 0.05$) 45min postmortem, and reduced drip loss 24h postmortem ($P < 0.05$). It concluded that maternal dietary VA supplementation had no effects on growth performance of offspring, and 21,600 IU/kg VA was required for optimal meat quality of offspring.

Keywords: Vitamin A, broiler breeders, broiler offspring, growth performance, meat quality

S1-0469 Replacing Efficacy of natusol® and antibiotic growth promoter on different parameters of broilers

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The objective of the present study was to investigate the effectiveness of a non-antibiotic growth promoter (Natusol) which is a combination of phytomolecules, direct-fed microbials (DFMs) and organic acids on performance, serum enzyme activities, immune response and visceral organ weights of commercial broiler chickens. A total of 300 day-old Ross 308 broiler chickens (mixed sex) were obtained from a local hatchery, weighed, and randomly allotted to 4 treatment groups with 5 replicates of 15 birds each. They received different treatments as basal diet (control); control plus 400 mg/kg bacitracin methylene disalicylate (BMD); control plus 0.05% Natusol (Natusol1) and control plus 0.1% Natusol (Natusol2). Chicks fed BMD resulted in maximum feed efficiency and a significant ($P \leq 0.05$) increase in the body weight, relative weights of liver, kidney and pancreas and Newcastle disease (ND), Infectious Bursal Disease (IBD) and Avian Influenza (AI) titers at d 42. Dietary inclusion of Natusol at the level of 0.1% into the basal diet proved its efficiency equal to BMD fed chicks, whereas addition of a probiotic at the level of 0.1% showed its applicability similar to the control group. It is concluded that the tested Natusol product at 0.1% but not 0.05% in the current study may be a suitable replacement for BMD in poultry diets.

Keywords: broiler chicks, immune response, performance, phytogenic feed additive

S1-0470 Effect of different levels of a multi-strain probiotic on performance, biochemical parameters, intestinal bacterial count and morphology and immune response of broilers, in comparison with antibiotic growth promoter

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To evaluate the compressional effect of different levels of a multi-strain probiotic with antibiotic growth promoter, 300 Ross 308 day-old broiler chicks were employed in a completely randomized design manner with 5 treatments and 3 replicates with 20 chicks each. Dietary treatments were 1) control basal diet, 2) basal diet with 0/45% Flavomycin antibiotic growth promoter, 3) basal diet with 0.0365% Primalac® probiotic, 4) basal diet with 0.0475% commercial Primalac® probiotic and 5) basal diet with 0.0593% of Primalac® probiotic. Result showed significant favorable changes among dietary treatments on body weight, feed conversion ratio, liver, the stomach (fore stomach), liver, cholesterol, triglyceride, ALP, HDL, LDL, albumin, total protein, ND, IBD, AI, bacterial counts of *E. coli* bacteria, coliforms, and salmonella, villus height, crypt depth and villous height to crypt depth ratio of small intestine. In conclusion, dietary different levels of Primalac® could reveal a similar impact on studied parameters of broilers and all 3 levels of Primalac® might be good replacer to AGPs.

Keywords: probiotic, performance, biochemical parameters, intestinal morphology, immune response, broilers

S1-0471 Effect of dietary trace mineral concentration and source (inorganic vs. organic) on production performance, eggshell quality and hatching performance in broiler breeders

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A total of 1080 32 wk-old breeders were used to determine effects of different concentrations of Zn, Cu, Fe and Mn, and trace mineral (TM) source [inorganic vs. organic (methionine hydroxy-analogue chelate, MINTREX®, Novus International, Inc)] on production performance, eggshell quality and hatching performance. The breeders were fed a common corn-soybean basal diet for 2 wks and experimental diets supplemented with 5 different levels of TM for 16 wks with 9 replicates per treatment (trt): 1) The control trt was supplemented with 80, 6, 100, and 65 mg/kg of Zn, Cu, Mn and Fe in sulfate forms; 2) Organic trt was supplemented with 48, 12, 48 and 50 mg/kg of chelated Zn, Cu, Mn and Fe-Gly; 3) Full adding organic trt supplemented with 16, 4, 16 and 2.5 mg/kg chelated Zn, Cu Mn and Fe-Gly on top of the control; 4) Half adding trt was supplied 50% Zn, Cu, Mn and Fe in sulfate form supplemented with 16, 4, 16 and 2.5 mg/kg chelated Zn, Cu, Mn and Fe-Gly; 5) Full adding inorganic trt supplemented with 16, 4, 16 and 2.5 mg/kg Zn, Cu, Mn and Fe in sulfate forms on top of the control. TM source did not affect laying rate in the experiment phase. The half adding trt and full adding inorganic trt reduced egg weight from breeders during 35-51 wks of age compared to the control, but organic trt and full adding organic trt did not differ from the control. Laying rate, broken egg rate, abnormal egg rate, eggshell intensity were not different among all trts during 35-51 wks. The mortality rate was higher in breeders that received the control diets compared with those received other diets ($P < 0.01$). Adding full dose of organic trace minerals increased ($P < 0.01$) the health rate and fertilized egg hatchability compared to trt adding the same dose of inorganic trace minerals. These results indicate that extra supplementation of chelated Zn, Cu Mn and Fe-Gly to the basal diets with commercial levels of inorganic trace minerals will decrease mortality and increase hatching performance.

Keywords: organic trace mineral, broiler breeder, performance, eggshell quality, hatching performance

S1-0472 Hematology and serum lipid profile of local toms fed four strains of *Lactobacillus* spp based probiotics

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A sixteen week study was conducted to determine the effect of four strains of *Lactobacillus* spp. based probiotics on the hematological indices and serum lipid profile of local toms. A total of 80 day old local male turkeys were randomly assigned to four treatments in a completely randomized design (CRD) with 20 birds per treatment. Treatments T2, T3 and T4 received probiotics of *L. delbrueckii* subspecies *bulgaricus*, *L. acidophilus* and *L. sporogenes* respectively at inclusion levels of 0.5ml/L in their drinking water for 3 consecutive days from 1 - 3, 10 - 12 and 21 - 23 days of age to achieve the recommended microbial concentration T1 served as control with no probiotics inclusion. Each treatment was replicated twice with 10 birds per replicate. Feed and water were provided ad libitum. Results showed that the effect of the different strains of *Lactobacillus* spp on hemoglobin, red blood cell count, white blood cell count, serum triglycerides, cholesterol, high density lipoproteins and low density lipoproteins were found to be significant ($P < 0.05$). Birds on probiotics inclusion had least values for white blood cell counts, low density lipoproteins, cholesterol and serum triglycerides with T4 (*L. sporogenes*) recording the most superior values. Birds on probiotics inclusion also had higher values for high density lipoproteins than the control with T4 (*L. sporogenes*) also recording the highest values as the birds aged. It was concluded that of the four strains of *Lactobacillus* sp based probiotics used in the study, *Lactobacillus sporogenes* supported superior haematological values with reduced lipid profiles.

Keywords: haematology, serum probiotics tom

S1-0473 Effects of coated benzoic acid and xylanase on growth performance, nutrients retention and plasma biochemical parameters of broilers fed wheat containing diets

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An experiment was conducted to evaluate the effects of coated benzoic acid (Provenia®, Novus International, Inc.) and xylanase on growth performance, nutrients retention and plasma biochemical parameters of broilers fed wheat containing diets (20% in starter and 30% in finisher diets). A total of 864 AA chicks were randomly distributed into battery cages (12 replicate cages per treatment) with 12 birds per cage at 1 d of age. The experiment was conducted as a 3 by 2 factorial arrangement with 3 levels of benzoic acid (0, 250 and 500 mg/kg) and 2 levels of xylanase (0 and 55 or 65 mg/kg from d 0-21 or 22-42). Acid-insoluble ash was used as an indigestible marker to determine nutrient retention. Pen was the experiment unit. Data were subject to 2-way factorial analysis to examine effect of benzoic acid, xylanase, and their interaction. In the absence of xylanase, benzoic acid at either 250 or 500 mg/kg increased final body weight; whereas no effect was seen when xylanase was present resulting in significant interaction ($P < 0.01$). Benzoic acid decreased plasma triglyceride content without xylanase; with xylanase supplementation benzoic acid increased the content accounting for significant interaction ($P < 0.01$). Xylanase increased plasma triglyceride content only in the presence of 500 mg/kg benzoic acid. Supplemental xylanase increased the retention of protein, calcium, energy ($P < 0.01$) regardless of benzoic acid level. Benzoic acid at 500 mg/kg and xylanase increased plasma glucose content on d 21. The inclusion of benzoic acid and xylanase did not affect FCR, death and culling rate, or retention of phosphorus and ash. These results indicate that in broilers fed diets containing 20-30% wheat, benzoic acid were beneficial in terms of improving growth performance, xylanase was effective in increasing nutrient utilization, and both modified plasma glucose and triglyceride content.

Keywords: broiler, coated benzoic acid, xylanase, wheat

S1-0474 Effects of Eimeria coccidial vaccination and dietary antibiotic alternative additives on the growth and carcass traits of male broilers

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The effects of commercial live Eimeria coccidial vaccination and dietary antibiotic alternatives on the growth and meat yield of commercial broilers were evaluated. A total of 1,120 Ross × Ross 708 male broilers were randomly allocated 80 floor pens (10 treatments, 8 replication pens/treatment, and 14 chicks/pen). A 2 × 5 factorial arrangement of treatments was used to determine the main and interactive effects of coccidial vaccination (with or without vaccination at hatch) and dietary additives [1. corn and soybean-meal basal diet, 2. basal diet + commercial antibiotics (bacitracin and salinomycin), 3. basal diet + probiotics (Bacillus subtilis), 4. basal diet + prebiotics (mannan-oligosaccharides and β-glucans), and 5. basal diet + probiotics + prebiotics]. On Day 15, all chicks were gavaged with a 20 dose of coccidial vaccine (live Eimeria oocysts) to simulate the coccidiosis challenge on commercial broiler production. Coccidial vaccination at hatch decreased Day 0-14 and Day 29-42 body weight gain (BWG) but did not affect Day 0-56 BWG. As compared to all other dietary treatments, antibiotic treatment decreased Day 0-14 and Day 15-28 feed conversion ratio, and increased feed intake and BWG from Day 15 to 28. However, no dietary effects were found on overall mortality or on growth performance from Day 29 to 56. In addition, there was no interaction between the vaccination and dietary additive treatments for growth performance or for any carcass parts, with the exception of breast yield. When compared to the individual antibiotic, prebiotic, and probiotic treatments, combination of probiotics and prebiotics increased the breast weights of the non-vaccinated broilers on Day 56. Coccidial vaccination at hatch did not alter the effects of the probiotic and prebiotic on overall growth. Additionally, antibiotics may promote the growth of broiler chicks at an early age, while the combined use of probiotics and prebiotics may facilitate their growth at a latter age.

Keywords: broiler, coccidial vaccination, prebiotics, probiotics, growth

S1-0475 Effect of protected sodium butyrate and nutrients' concentration on broilers gut health

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This trial was conducted to compare the effect on gut health, of sodium butyrate protected with sodium salt of palm fatty acids distillates, in diets for broiler chickens with different nutrient concentration. A 2 × 2 factorial design was performed with two basal diets based on maize and soybean meal: S (standard nutrient diet) and L (low nutrient diet) with a reduction of 60 Kcal/Kg of ME and 2.3% lower concentration of amino acids; with (Y, additive addition) or without (N, no additive addition) protected sodium butyrate at 1 kg/t. A total of 160 one day old broilers were randomly distributed to 4 treatments with 4 pens of 10 birds. At 21d, 1 chick per pen was killed and GIT were removed for the analysis of gut microflora (Lactobacillus, E. Coli and Coliforms) and development status of ileum and caecum epithelium. Data were analyzed with two-way ANOVA using the GLM procedure of SAS. Lactobacillus tended to be higher in SN group ($P < 0.10$) in ileum respect to the other treatments. No more differences were observed in Coliforms nor in E. Coli in ileum. The addition of additive showed a significant decrease in Coliforms (5.24×10^8 vs 1.44×10^8 CFU/g) and E. Coli (4.30×10^8 vs 9.57×10^7 CFU/g) in caecum. The addition of additive tended to increase villus height in a 9%, crypt depth in an 8% and increased mucosal thickness (669.5 vs 619.0 μ m; $P < 0.05$). The reduction in nutrients' concentration increased villus height (518.2 vs 470.1 μ m; $P < 0.05$), tended to reduce crypt depth (-6%) and increase mucosal thickness (6%). There was an increase in V:C ratio due to the reduction in nutrients' concentration (3.57 vs 3.05 ; $P < 0.05$). It can be concluded that the addition of protected sodium butyrate in diets for broilers at early stages induce a favorable effect on gut health, taking into account the reduction on bacterial populations in caecum and the improvement in intestinal epithelium. It might influence later stages improving the intestinal function and performance parameters.

Keywords: protected sodium butyrate, energy, amino acids, gut health

S1- 0476 Effect of product bioprocess shrimp waste as nutrient concentrate in the ration on characteristic carcass native chicken starter period

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Waste-product frozen shrimp processing industry is contains 43.41% crude protein, 18.25 percent crude fiber, 7.27 percent crude fat ,5.54 percent calcium, 1.31 percent phosphorus , and the gross energy 3892 kcal/kg, but also contains chitin 20:11%. One effort to convert organic material into useful new products is to use microbes through bioprocess.. Bioprocess waste- product shrimp namely nutrient concentrate, can be done through the stages deproteinated using *Bacillus licheniformis*, and demineralized with *Lactobacillus* sp. and terminated by *Saccharomyces cerevisiae* have a better protein digestibility value. The aim of the research was to evaluate characteristic of carcass native chickens to using nutrient concentrate in the ration. 150 DOC native chickens were raised in cages until eight weeks old. This experiment was conducted completely randomized design , six nutrient concentrate levels in the ration, namely R0 = basal ration without nutrient concentrate with (crude protein 15%, ME 2750 Kcal/kg), R1= ration contained 5% nutrient concentrate R2= ration contained 10% nutrient concentrate), R3= ration contained 15% nutrient concentrate , R4= ration contained 20% nutrient concentrate , and R5 = standard ration high protein without nutrient concentrate (crude protein 18%, ME 2750 kcal/kg) and repeated five times. Data were analyzed using analysis of variance and Duncan,s multiple range test. Variable analysis were final body weight, percentage of carcass , cholesterol and protein content of native chicken starter period. The results showed that treatment using nutrient concentrate in the ration was no significant effect on carcass percentage, but was significant effect on cholesterol and protein carcass native chicken.. The conclusion of experiment that by giving 10% nutrient concentrate in the ration gave the best of carcass quality and gave the same response with the standard ration (ration of 18% protein content).

Keywords: bioprocess product, shrimp waste, nutrient concentrate, carcass

S1- 0477 Selenium supplementation improves broiler growth under tropical conditions

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Hot climate conditions lead to poor broiler performances that are partly associated with oxidative stress. Through its functions into selenoproteins, appropriate selenium (Se) status may improve animal growth under environmental challenges such as tropical conditions. This study aimed to evaluate the effect of different Se sources fed to broiler chickens raised under tropical conditions (Thailand). One thousand and twenty four newly hatched male chicks (Arbor Acres Plus) were randomly allocated to four treatments x 16 replicates of 16 birds. During starter (0-16 days) and grower (21-35 days) periods animals were fed with standard corn-soybean meal diet differing only in Se source and level as follow: NC: negative control Se-unsupplemented; SS-0.3: supplemented with 0.3ppm Se as sodium selenite; SO-0.1 and SO-0.3: supplemented with 0.1 or 0.3ppm Se as hydroxy-selenomethionine (OH-SeMet). Average temperatures (T°C) and relative humidity (% RH) were representative of mild tropical conditions: period 0-7 days, 31.8°C-73% RH; period 7-21 days: 28.2°C-84% RH; period 21-35 days: 26.8°C-90% RH. At 35 days, body weight of NC group (2645g) was lower than SO-0.1 (2698g) and SO-0.3 (2702g) groups ($P<0.05$); SS-0.3 animal body weight (2677g) was intermediate and not significantly different from other groups ($P>0.05$). Animal feed intake were numerically lower in the NC and SS-0.3 groups compared to SO-0.1 and SO-0.3 groups with 3813g and 3817g compared to 3864g and 3863g, respectively. This reduced feed intake may be associated with a lower resistance to those environmental conditions. Feed conversion ratio was only numerically improved in Se supplemented groups compared to NC group. Those results demonstrates the importance of a Se supplementation for optimal animal growth under challenging conditions. Study at a larger scale might reveal the advantage of organic OH-SeMet compared to inorganic selenium using supplementation level as low as 0.1 ppm Se.

Keywords: selenium, hydroxy- selenomethionine, broiler chicken, tropical

S1- 0478 Vitamin E and selenium source or level effect on broiler breeders antioxidant status and performances

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Eight hundred female broiler breeders (Cobb 400) at 51 weeks of age were divided into four groups x 10 replicates x 20 birds, in a 2 x 2 factorial design: two alpha tocopherol levels, 50 or 200 ppm Vit E and two selenium sources, sodium selenite (SS) or hydroxy-selenomethionine (OH-SeMet) at 0.3 ppm Se. Those four treatments were distributed from week 51 of animal age to week 64. Laying performances were recorded throughout this period; eight eggs per replicate were collected at week 54, week 58 and week 62 for egg quality measurements. One animal per replicate was blood sampled for antioxidant parameters at the same times (week 54, 58 and 62). Finally, on week 58 and week 62, eggs were collected and incubated at room temperature for 5 or 10 days. Egg production, feed conversion ratio and egg quality parameters were not affected by the different treatments throughout the study period ($P > 0.05$). Other results indicated main improvements caused by Se sources rather than Vit E levels. Glutathione peroxidase (GPx) and glutathione reductase (GR) activity were numerically enhanced in OH-SeMet groups compared to SS at week 54, 58 and 56, the enhancement of GR activity reach significance at week 62 ($P < 0.05$). Superoxide dismutase activity appeared significantly improved in OH-SeMet groups compared to SS groups at week 58 ($P < 0.05$) and only numerical improved for the two other periods. Storage time of eggs prior incubation significantly lowered hatchability ($P < 0.05$), but results indicated that OH-SeMet groups significantly maintained a better hatchability at week 62 compared to SS groups: 81.2% and 78.3%, respectively ($P < 0.05$). Those improvements were observed through several antioxidant parameters at the animal level using OH-SeMet compared to SS; hatchability was also maintained at a higher level with OH-SeMet an indication that such an organic selenium source can contribute to improve antioxidant status of the animal and influence its performance.

Keywords: selenium, hydroxy- selenomethionine, broiler breeders, antioxidants

S1-0479 Selsaf, a source of selenium improving zootechnical performance

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Although mostly known for its toxicity, selenium has become an indispensable trace element for the poultry industry as it improves hatchability, fertility and embryonic development. Selenium concentrations in soil and plants are currently insufficient in large parts of the world; therefor supplementation of animal feed with this trace element is essential. Consequently, selenium-enriched yeasts have been added to poultry feed for many years as the selenium present is highly bioavailable and has the capacity to be stored in animal proteins under the form of seleno- methionine (SeMet). As recently synthetic forms of SeMet were brought to the market, the aim of the trial was to compare several different forms of selenium supplements in laying hens. At the start of the experiment 1260, 17-week-old laying hens from the Bovans brown genotype were randomly divided into 3 groups, each containing 14 replicates. Laying hens were supplemented with either; inorganic selenium (SS), Selsaf or synthetic seleno-methionine (synth SeMet) at a 0,2ppm selenium inclusion rate. Subsequently, from the start of lay, laying hen performance parameters including; feed consumption, feed consumption/egg, laying intensity were recorded during 32 weeks in 8 periods lasting for 4 weeks. Additionally, mortality was recorded daily while selenium deposition in liver and blood glutathione peroxidase (GPx) activity was measured at the end of the experiment. Although almost no differences were detected in GPx activity, or in the selenium deposition in the liver, the results clearly indicate that Selsaf highly increased the survival rate of the laying hens during the experiment. Additionally, Selsaf strongly improved the feed consumption per egg and the laying intensity without affecting the average weight of the eggs. In conclusion, this trial clearly shows that Selsaf is a highly bioavailable source of selenium with a high capacity to improve the health status and production capacity of the animal.

Keywords: glutathione peroxidase, yeast, performance, selenium

S1-0481 Content and bioavailability of carotenoids in corn distillers solubles oil for skin pigmentation and plasma concentration in broilers

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Corn distillers solubles oil (CDSO) is a co-product of the corn ethanol distillers process and may have value as a carotenoid source to enhance pigmentation of broiler skin besides its dietary energy value. The variability in quality and content of carotenoids in CDSO produced by 11 ethanol plants was surveyed, and the carotenoid bioavailability based on skin pigmentation and blood plasma carotenoids in broilers was determined. The mean \pm std fat quality indicators and carotenoid composition of the CDSOs were determined to be 1.5 ± 0.2 MIU, 0.2 meq/kg PV, 20.4 ± 0.2 p-anisidine, 49.6 ± 26.4 meq/kg 20h AOM, and 230 ± 47.7 ppm total carotenoids (96.7 ± 21.1 trans-lutein, 75.53 ± 17.9 trans-zeaxanthin, 35.9 ± 8.96 cis-lutein/Zeaxanthin, 12.1 ± 4.43 alpha-cryptoxanthin, and 9.4 ± 1.86 beta-cryptoxanthin). CDSO of average total carotenoid content was used in the bioavailability trial and compared to a commercial pigment extract product. Basal diets containing white corn and soybean meal were supplemented with 0, 25, 50, 75, and 100 % CDSO added in place of 4% soybean oil. To serve as positive control, Yellow Pixafil Liquid-LZ (Alcosa Biotech) supplied the same level of total carotenoids as the 100% CDSO diet. Ross 344X708 chicks were randomly assigned among 7 reps/treatment (10 chicks/rep) with feed and water provided ad libitum. At 51 d, paw and breast skin yellowness (b^*) was evaluated by a minolta colorimeter, and plasma carotenoid content was determined. Paw and breast skin yellowness (b^*) and plasma carotenoid content increased linearly as dietary CDSO level increased ($P < 0.001$). Relative to the commercial pigment source, the carotenoids in CDSO were determined to have $>90\%$ bioavailability. While total carotenoid content of CDSO varies among ethanol plants ($\sim 20\%$ CV), the bioavailability of CDSO carotenoids is similar to a commercial marigold pigment product based on skin pigmentation.

Keywords: carotenoids, skin pigmentation, broilers, corn distillers solubles oil

S1- 0482 Effects of quercetin on blood antioxidant parameters and liver protection of broiler chicks induced oxidative stress by tert-BHP

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In order to evaluate the effects of quercetin on broilers blood antioxidant parameters and liver protection, an experiment was performed by using 240 ROSS 308 male broiler chicks. The experiment was accomplished in completely randomized design including 4 treatments with 4 replication and 15 observations in each replicate. Experimental groups consist of: (1) control, (2) quercetin, (3) tert-BHP, and (4) tert-BHP with quercetin. From 14th day in the quercetin and tert-BHP with quercetin groups birds were administered 15 mg/kg body weight of quercetin once daily via oral gavages. Three days before each blood collecting, the birds belonging to oxidative stressed experimental group received 0.2 mmol/kg body weight tert-BHP intraperitoneally. The results showed that 0.2 mmol/kg body weight t-BHP caused an increase in Malondialdehyde levels as a reliable indicator of lipid peroxidation. Total antioxidant capacity was decreased in oxidative stressed experimental group. Activities of superoxide dismutase and glutathione peroxidase were increased in the oxidative stressed experimental group (3 and 4). Aspartat aminotransferase and alanine aminotransferase were increased during oxidative stress which can related to damage to hepatocytes. These results indicated that quercetin supplementation improved oxidant/antioxidant balance of oxidative- stressed broilers and offers quercetin as a helpful supplement in oxidative stress condition.

Keywords: tert butyl hydroperoxide, quercetin, oxidative stress, liver protection, broilers

S1- 0483 Evaluation of the efficacy of xylanase and arabinofuranosidase enrichment of a multi-enzyme complex on metabolisable energy value of corn-soybean meal- and wheat-soybean meal-based diets

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Plant cell wall degrading enzymes (enz) are key technologies in animal feed industries. The present study describes the effect of an endo-xylanase (Xyl; GH11) and an arabinofuranosidase (Abf; GH54) on the digestibility of different raw materials as estimated by an in vitro method. The effects of an enrichment of a multi-enz complex with these two enzymatic activities were also determined by an in vivo digestibility tests. In in vitro trial, the effect of Xyl and Abf alone or combined was measured on dry matter digestibility (dig DM) of wheat, corn and corn distiller. The highest effect of Abf was observed on corn and corn distillers (respectively 5.7 and 14.8% improvement), which are characterized by high level of substitution of xylose backbone with arabinose. Xyl improved wheat dig DM by 3.8% ($P < 0.001$) and corn distillers dig DM by 13.3%. Combination enz further improved these values to 8.9 and 15.9% ($P < 0.001$). In vivo digestibility trials with broilers (12-22 day) were conducted by using corn-soybean meal or wheat-soybean meal-based diets supplemented with either multi-enz (Rovabio Excel) or Xyl and Abf enriched preparation (Rovabio Advance) at their commercial doses. The apparent metabolisable energy (AME) were measured. Compared to control without enz addition, both enz preparations significantly improved AME of wheat-soybean meal-based diet at a similar rate: 90 and 100 Kcal/kg DM for Excel and Advance, respectively. On corn-soybean meal-based diet, Rovabio Excel numerically improved AME by 59 kcal/kg DM ($P < 0.10$) and Rovabio advance significantly increased AME by 95 kcal/kg DM ($P < 0.05$), respectively. In conclusion, both in vitro and in vivo studies showed that enrichment in Xyl and Abf activities was of great interest in dietary fiber degradation especially for corn. This observation could be related to the high ability of Abf to increase the potential activity of Xyl, as corn presents a high degree of ramification of xylose backbone with arabinose.

Keywords: arabinofuranosidase, energy, enzyme, xylanase

S1- 0484 Replacement value of maize with different levels of sun-dried cocoyam (*Xanthosoma Sagittifolium*) as energy source on the performance of broiler finisher

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Limitation in poultry production imposed by scarcity of maize and competition with human consumption in developing countries like Nigeria have forced many farmers into employing alternative sources of energy for poultry feeds. This study was carried out to determine the nutritive value of sundried wild variety of cocoyam (*Xanthosoma sagittifolium*) when it is used to replace yellow maize as source of energy in the diets of finisher broilers, at levels of 10%, 15%, 20% and 25 % respectively. Sun dried xanthosoma sagittifolium (Tannia) contained 9.26% moisture, 3.60% ash, 3.70% crude protein, 7.80% crude fiber, 0.11% ether extract, 75.58% NFE, respectively. One hundred and twenty (120) unsexed Finisher Anak broilers at 5 weeks of age were assigned to 5 experimental diets using Completely Randomized Design. Each treatment had 24 finisher broiler chicks which was divided into three replicates with 8 birds per replicate. The experiment lasted for 4 weeks. Feed intake and body weight gain were recorded weekly. In the finisher phase, the group on 15% sundried cocoyam tuber meal (diets), replacing maize recorded the highest body weight gain, better feed intake and feed conversion ratio which were significantly ($P < 0.05$) different from other groups. Diet 2 (15% SCYM) appeared to be the most economical to use for finisher broilers because the cost of production per Kg of broiler was N361 versus N 373.96 for the control diet. The internal organs expressed as percent of the live weight were not affected by the treatment. The finisher broilers on diet 2 recorded the highest dressing out percentage of 91.91 while the group on diet 5 recorded the lowest dressing percentage of 88.18. The study showed that sun dried cocoyam tuber meal (*xanthosoma sagittifolium*) could be used up to 15% in the diet of finisher broilers without affecting feed intake, weight gain and feed conversion ratio.

Keywords: performance, replacement, energy source, maize, cocoyam, broiler finisher

S1-0485 Application of chicken egg yolk immunoglobulins in land and aquatic animal diseases control

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Chicken egg yolk immunoglobulins, referred to as immunoglobulin Y (IgY), has recently attracted considerable attention, as it possesses unique advantages compared with mammalian IgG by their cost-effectiveness, convenience and high yield. Specific IgY antibodies has offered potential prophylactic and therapeutic value in controlling animal diseases. Various research groups reported that oral administration of specific IgY antibodies was an effective means of controlling enteric infections of either bacterial or viral origin in piglets, calves, rabbits, poultry and fish. In our previous work, successful applications have been reported including studies which demonstrate the ability of oral passive immunotherapy with IgY to control piglet diarrhoea caused by enterotoxigenic *Escherichia coli*, bovine *Staphylococcus aureus* mastitis. As well, specific IgY has been studied against white spot syndrome virus in cultured shrimp, septicemia caused by *Aeromonas hydrophila* in crucian carp, and *Vibrio splendidus* infection in sea cucumber. IgY is a promising alternative to antibiotics for passive immunotherapy application, however, its application is limited by its sensitivity to gastric conditions. Therefore, it is highly necessary to develop an effective delivery system to protect orally administered IgY from the harsh gastrointestinal environment enroute to the infection site in the intestine. The microencapsulation technique enables a large proportion of IgY to remain bioactive in simulated gastro-intestinal tract environment, providing a more effective delivery of specific IgY. This review presents an overview of potential immunotherapy with specific IgY for the prevention and treatment of land and aquatic animal diseases and speculates on the future of IgY technology.

Keywords: egg yolk immunoglobulin; IgY; terrestrial; aquatic; disease control

S1-0486 Passive immunization of turbot (*Scophthalmus maximus*) with chicken egg yolk immunoglobulins (IgY) against *Edwardsiella tarda* infection

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Oral delivery of chicken egg yolk immunoglobulins (IgY) serve an interesting opportunity for controlling bacterial diseases in aquaculture and providing passive immunization of aquatic animals. This study evaluated the protective effect of IgY against *Edwardsiella tarda* infection in turbot. IgY was isolated from egg yolks laid by hens immunized with inactivated *Edwardsiella tarda*. Purified IgY was shown to inhibit the growth of *Edwardsiella tarda* in vitro in a dose-dependent manner at concentrations ranging from 1 to 10 mg/mL. And the presence of specific IgY induced a attenuation of biofilm formation and the hydrophobicity at the surface of bacteria was decreased significantly ($P < 0.05$). High survival rate (87.5%) of turbot was observed in groups treated with anti-*E. tarda* IgY after bacterial infection, compared with those treated with non-specific IgY (31.25%) or no administration (18.75%). As well, the bacterial burden in liver, spleen, kidney and intestine was significantly ($P < 0.05$) lower in turbot treated with specific IgY than those treated with non-specific IgY. Moreover, preventive protection was shown in groups diet with 2% IgY powder and 10% egg yolk powder, which provided 75% and 62.5% survival rate, respectively and had better performance compared to 2% egg yolk powder group and 0.2% sIgY powder group and dramatically difference compared to the negative control group ($P < 0.05$). These results suggest that passive immunization with specific IgY by oral intubation may provide a potential treatment for *Edwardsiella tarda* infection in turbot.

Keywords: *Edwardsiella tarda*, passive immunization, turbot, egg yolk immunoglobulins, IgY

S1- 0487 Effects of dietary methionine on performance, egg quality and glutathione redox system in egg-laying ducks

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In this study, six dietary DL-methionine (Met) levels (2.5 (control), 3.0, 3.5, 4.0, 4.5 and 5.0 g/kg) were tested to estimate the dietary Met requirements of Longyan ducks from 19 to 46 wk of age, and to investigate its effect on the glutathione redox system. In total, 1080 Longyan ducks aged 19 wk were allocated randomly to the six dietary treatments, where each treatment comprised six replicate pens with 30 ducks per pen. Met had no effects on egg production, yolk weight, yolk colour or the glutathione redox system, but the egg weight, egg mass and feed conversion ratio (FCR) were improved significantly by dietary Met supplementation. As the dietary Met concentration increased, the eggshell thickness and breaking strength decreased significantly, whereas the albumen weight increased significantly. According to broken-line regression analysis, the optimum Met requirements for egg weight, egg mass, FCR and albumen weight are 686, 661, 658 and 731 mg/duck-d, respectively, with a dietary crude protein level of 170 g/kg.

Keywords: methionine; laying ducks; egg quality; glutathione redox system

S1- 0488 Utilization of DL-methionine Hydroxy Analogue Calcium Salt, DL-methionine and L-methionine in grower broilers

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To evaluate the relative bio-efficacy (BE) of DL-Methionine Hydroxy Analogue Calcium Salt (MHA-Ca), DL-Methionine (DLM) and L-Methionine (LM) at the grower stage (22-42d), a total of 2080 21-d-old healthy Rose 308 male broilers were selected and randomly allotted to 13 treatments with 8 replicates of 20 birds and fed in floor pens (2×1m) with rice husk as litter. A corn-soybean meal-pea protein concentrate-based grower basal diet was formulated deficient in methionine and cystine. Four graded levels of DLM or LM (0.05, 0.10, 0.14, and 0.24%) or equimolar MHA-Ca were supplemented on top of the basal diets to create 13 experimental diets. Diets were in pellet form. The broilers had access to feed and water ad libitum. The average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were measured. At 44d, 1 birds per pen with body weight close to the average were selected to determine the carcass performance. Non-linear multi-exponential regression as suggested by Littell et al. (1997) was applied using the NLIN procedure of SAS. The relative bio-efficacy of LM and MHA-Ca over DLM was calculated by coefficient ratio of LM and MHA-ca to DLM. The results showed: based on equimolecular addition, the BE of LM was 94.9% and 104.6% for ADG and FCR at 22-42d, while the BE of MHA-Ca was 72.3% and 93% respectively. Based on the carcass performance, the BE of LM was 108.6% for breast meat weight and 113.1% for breast meat yield, while the BE of MHA-Ca was 87.2% and 75.3%, respectively. In conclusion, the average BE of LM was 99.8% for growth performance and 110.9% for carcass performance, while that of MHA-Ca was 82.7% and 81.4%, respectively. On a product basis, MHA-Ca was 68.9% as efficacious as DLM. These values, especially MHA-Ca, were numerically much higher than those got from our early experiment which was done during 10-42d. However, a conclusion cannot be easily made before the values of early stage broiler were clear.

Keywords: DL-Methionine hydroxy analogue calcium salt; DL-Methionine; L-Methionine; grower Broilers

S1- 0489 Cytokine gene expression in chicken related to barrier functions of the intestinal mucosa following with microencapsulated *E. faecalis* and the extract of *Camellia oleifera* seed

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The aim of this study was to investigate the effects of dietary supplemented with microencapsulated *E. faecalis* and the extract of *Camellia oleifera* seed, independently or in combination, on growth performance, intestinal morphology, gene expressions related to barrier functions of the intestinal mucosa in broiler chickens. 160 day-old Arbor Acres male broilers were randomly allotted to 4 treatments with 8 replicates of 5 chicks each in a 2×2 factorial arrangement, evaluating two levels of MEF (0 or 10 g/kg of diet), and two levels of ECOS (0 and 300 mg/kg of diet). Data were analyzed by two-way ANOVA of SPSS 18.0. Differences were considered statistically significant at $P \leq 0.05$. Birds fed MEF (2.4×10^7 cfu /g diet) had lower FCR ($P < 0.05$) from days 21 to 42 and greater ADG ($P < 0.05$) from days 0 to 42 than the control birds. Dietary MEF supplementation significantly increased the villus height, and villus height to crypt depth ratio in jejunum and ileum of broilers at 21 and 42 day. Birds fed MEF had greater ($P < 0.05$) gene expression of MUC2, occludin and claudin-1 in jejunum and ileum mucosa than the control birds at 21 and 42 day. Birds fed ECOS containing 300 mg /kg of diet had slightly beneficial effects on growth performance and intestinal barrier functions. Besides, the results showed that there was no significant interaction between MEF and ECOS on growth performance, mRNA expression of MUC2, occludin and claudin-1 in jejunum and ileum mucosa. In conclusion, the current results indicated that MEF can improve growth performance and enhance intestinal barrier functions by improving its intestinal morphological development and up-regulating the gene expression of MUC2, occludin and claudin-1.

Keywords: microencapsulated *Enterococcus faecalis*, extract of *Camellia oleifera* seed, growth performance, intestinal barrier function, gene expression, broiler

S1-0490 Recent advances in sunflower seed meal as an alternate source of protein in broilers: An overview

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Broiler production in developing countries often suffers from an inadequate supply of local high quality protein feed ingredients. Increased production of sunflower seeds (*Helianthus annuus* L.) for oil will provide more meal that could be added to broiler diets to provide protein and offset the need for soybean often unavailable due to cost. Sunflower seeds, initially cultivated before soybeans, were one of the first plants cultivated in the Americas by Indian tribes. Presently, due to adaptive capabilities in various climatic and soil conditions, sunflower seeds are grown worldwide. Sunflower seed meal (SFSM), a by-product from oil production for human consumption, varies in quality due to variations in oil extraction processes. However, investigators reported that SFSM can replace up to 2/3 of the soybean meal protein in the starter and finisher diets of poultry. In this comprehensive review, we have examined the quality of SFSM as related to (1) the effect of processing and (2) as a high quality feed ingredient for broilers.

Keywords: recent advances, sunflower seed meal, alternate protein source, broilers

S1- 0491 The nutritional evaluation and utilization of hatchery waste egg meal for laying hen

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The purpose of this study was to compare the effect of hatchery waste egg meal (HWEM) processed by superior conditions acquired from previous research with that of fish meal on laying performance. Seven batches of HWEM were produced, mixed and analyzed for its chemical composition and apparent metabolisable energy corrected to nitrogen equilibrium (AMEn) to prepare four diets containing 0, 4, 8 or 12% of HWEM, respectively. Besides, feed containing 2% of fish meal was also prepared. One hundred single comb white Leghorn hens at 23 weeks of age were assigned randomly into five groups and fed with these diets for 18 weeks. The body weight, egg production, egg weight, shell strength, and feed intake of the layers and the chemical analyses of the diets were measured. The results showed that the digestibilities in vivo of protein, fat, calcium, phosphorus and dry matter of HWEM were 78.5, 60.1, 40.3, 37.2 and 85.7% in cockerel and 88.6, 77.8, 90.2, 62.5 and 87.9% in laying hen, respectively, and its AMEn was 3378.4 kcal/kg. There was no significant difference in the criteria of the layers, including body weight, intake, feed efficiency and egg weight. The HWEM is a good source of calcium because the shell strength from hens treated with feed containing 4, 8 or 12 % was significantly higher than that of the 0% and fish meal groups, whereas no significant difference existed in this criterion between these HWEM groups. HWEM can enhance shell strength and no adverse effects on egg quality characteristics.

Keywords: hatchery waste egg, apparent metabolisable energy corrected to nitrogen equilibrium, egg quality, Laying hen

S1-0492 Partial or total replacement of soybean oil by black soldier pupae fat in broiler diets. Part 1: Effect on growth and slaughtering performances

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Aim of this study was to determine the effect of partial or total replacement of soybean oil by black soldier pupae fat (BSPF) in broiler diets on animal growth performance. A total of 150 male broiler chicks (Ross 308) at 1-d of age were randomly allotted to 3 dietary treatments, each consisting of 5 pens as replicates (10 chicks/pen). A basal diet based on corn meal, soybean meal and soybean oil was formulated and served as control group; the 50 and 100% replacement of soybean oil by BSPF formed the two treatment groups. All diets were formulated to be isonitrogenous and isoenergetic and were split in 2 phases: starter (1-21 d) and finisher (21-35 d). Daily feed consumption, daily weight gain and feed conversion ratio were determined for each diet phase and for the overall trial period. At 35 d of age, final body weight was recorded and 15 chicks (3 chicks/pen) from each feeding group were slaughtered and dissected to determine their carcass yields. The weights of liver, spleen, heart and abdominal fat were also recorded. Differences among groups were tested by one-way ANOVA, followed by Duncan's post-hoc test. Significance was declared at $P < 0.05$. Growth performance, carcass yield and organs weight were not influenced by the partial or total replacement of soybean oil by BSPF. In conclusion, BSPF seems suitable to be used in starter and finisher broiler diets without any negative growth impact.

Keywords: broiler chicken, black soldier pupae fat, insects, performance

S1- 0493 Effects of supplementing broiler diets low in crude protein with lysine

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Protein is generally considered as one of the major cost components of the poultry diets. Therefore, importance of using appropriate amounts of balance dietary protein is high priority issue for several reasons like feed efficiency, cost and environmental concerns. The objective of the study was to evaluate the effects of lysine supplementation in low protein diets on the performance of broiler during the period of 11-24 d. Three primary diets were contains 21, 19 and 17% CP. In each of these diets were supplemented with additional two levels of lysine i.e. 1.2 and 1.3%. A total of 600 Ross 308 male broilers were fed to 6 treatments with 5 replications (20 birds per pens) during grower period. It can be concluded that reduction of CP in the grower period had a significant effect on the live performance. Decreasing CP levels lower than 19% significantly decreased body weight gain and increased the feed conversion ratio. Adding the lysine to the low CP diets significantly improved the performance but did not completely overcome the adverse effects of the low CP diets.

Keywords: broiler, low protein, lysine, supplementation

S1-0494 Eggs may reduce vitamin D deficiencies in the human population

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Eggs are a naturally good food source of vitamin D containing both vitamin D3 and the more potent 25-hydroxyvitamin D3 (25(OH)D3). Epidemiological surveys confirm a global chronic vitamin D insufficiency in the human population particularly in China and India. It is proposed that eggs could provide a natural and economical food source of vitamin D, and thereby help alleviate global vitamin D deficiencies in children and adults. In order to confirm this hypothesis, a total of 17,000 free range hens were fed two levels of vitamin D3 (6,000 and 10,000 IU/kg) in combination with one level of 25(OH)D3 (69 µg/kg) for four weeks. Egg yolks were analysed for their vitamin D3 and 25(OH)D3 content, as well as the effect of cooking on vitamin D content. Egg production, egg mass feed efficiencies and mortalities were recorded. The concentration of vitamin D in egg yolk was significantly increased at the higher level of vitamin D3 inclusion, with average vitamin D content being 360 IU per egg. There were no significant differences in egg mass, mortality or feed efficiency. It was possible to produce a single egg which would contribute significantly to the recommended daily requirement of vitamin D for children and adults. The feeding of higher levels of vitamin D to laying hens can provide a positive solution to the chronic problem of vitamin D insufficiency in the human population, at minimal cost and without detrimental effect on production parameters.

Keywords: vitamin D, egg, deficiency

S1-0495 Enrichment of broiler chicken meat with n-3 PUFA using linseed oil and fish oil and their influence on performance, carcass traits, serum lipid profile and sensory characters of meat

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In a 42 day trial, 180-day old broiler chicks were randomly distributed into 3 dietary groups (10 replicates with 6 birds in each) to study the effect of n-3 fatty acid (FA) rich linseed oil (LO) and fish oil (FO) dietary incorporation on performance, carcass traits, serum lipid profile (on day 35), fatty acid composition and sensory attributes of meat. Corn-soybean based control diet was prepared using sunflower oil (SFO). Subsequently, two test diets were formulated by replacing SFO with LO and FO on weight basis. On day 43, 8 birds per treatment were slaughtered to study carcass traits, sensory characters and FA composition. The birds performance and carcass traits (dressing yield, breast yield, liver, heart, and gizzard) were not influenced by oil sources but abdominal fat deposition lowered ($P<0.01$) with LO or FO supplementation compared to SFO. Serum total and LDL cholesterol concentrations lowered ($P<0.05$) and HDL cholesterol concentration increased ($P<0.01$) with LO or FO compared SFO. LO or FO supplementation increased ($P<0.05$) total n-3 PUFA, total PUFA, and lowered ($P<0.05$) the total n-6 PUFA, total SFA and n-6/n-3 ratio without affecting the sensory (appearance, flavour, juiciness, tenderness and overall acceptability) attribute of meat. Moreover, higher ($P<0.01$) C20:5 n3 and C22:6 n3 FA depositions in meat noticed with FO supplementation compared to LO. Whereas, highest ($P<0.05$) C18:3 n3 FA deposition in thigh meat recorded with LO supplementation. This could be concluded that dietary incorporation of LO or FO at 3% level could enrich the meat with n-3 FA and lowers the n-6:n-3 ration without affecting the broilers performance and sensory characters of meat.

Keywords: broiler chickens, fatty acid composition, n-3 pufa oils, performance, sensory characters of meat

S1-0496 Effect of feeding of probiotic containing naturally isolated culture of Lactobacilli in overall growth performances and controlling early chick mortality in commercial broiler chicks

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Two hundred and twenty five Cobb 500 FF male broiler chicks were allocated in three groups such as control, Treatment - 01 & 02 and each group containing seventy five chicks representing twenty five chicks in each replicate. Control group was subjected to drink normal potable water and Treatment - 01 group was subjected to preventive antibiotic medication in oral route from day - 01 to day - 05 and followed with normal potable water and Treatment - 02 group was subjected to drink naturally isolated culture of Lactobacilli in liquid solution containing Lactobacilli count at 2.7×10^6 CFU per ml and mixing to sanitized normal potable water from day 01 to 40 days. All groups were subjected to feed ad libitum with standard broiler diet in mash form without any antibiotic growth promoters (AGPs). The results have shown that mortality %, body weight, feed conversion efficiency at third day, seventh day and fortieth day (Market Age) and broiler production efficiency index (BPEI) significantly differed in Treatment- 02 group ($P<0.05$) in comparison with both control and treatment -01 groups respectively. These results conclude that naturally isolated Lactobacilli culture could be effectively used for the achievement of target performances in commercial broiler production in respect to control of early chick mortality and improving growth.

Keywords: probiotic, lactobacilli, commercial broilers, feed conversion efficiency

S1- 0497 Effect of diets containing different medium chain fatty acids (MCFA) on growth performance and ileum bacterial load of broiler chickens fed two basal diets

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MCFA are a type of saturated fatty acids which consists of 6 to 12 carbon atoms mainly found in some oils or fats such as coconut oil and palm kernel oil. This study aimed to test the effect of supplementation of caproic (C6), caprylic + capric (C8+C10) and lauric (C12) acids in the feed on performance, viscosity and bacterial load of ileal content of broilers fed either a viscous (V) or a non-viscous (NV) diet. 1200 day old Ross 308 broilers were allocated in 10 treatments with 6 replicates of 20 birds, males and females in separated pens (60 in total, 2 m2 each). A 5 (test products) x 2 (basal diets) x 2 (sexes) factorial design was used. The experiment treatments contained: i) a control diet (no added MCFA); then the control diet + 1% of either: ii) C6, iii) C8+C10, iv) C12 or v) a mixture of C6, C8+C10 and C12 (0.33% each) supplemented either in a V or NV diet. MCFA were added to the control diet at expense of 0.5% of soybean oil and 0.5% of animal fat. Performance parameters between d 7-28 of age were determined. Ileal digesta samples were collected from 2 birds per pen at d 24. Total bacteria (TB), total Lactobacillus (TL) and total Lactobacillus acidophilus (TLA) loads were determined through individual DNA extraction and qPCR. Data were analysed by ANOVA using GenStat. No effect of MCFA on body weight gain was observed. C6 improved feed conversion ratio (FCR) in both basal diets and reduced numerically TB load in the V diet. C8+C10 improved FCR in both basal diets and reduced TB and TL loads in the V diet. C12 improved FCR in the V diet and reduced TB load regardless the viscosity of the diet. Combined MCFA did not have a synergistic effect on FCR, but it had a significantly better FCR and reduced TB load in the V diet. In conclusion, inclusion of 1% of C6 and C8+10 improved feed efficiency in the NV diet whereas all MCFA improved FCR in the V diets, caused probably by their antibacterial property.

Keywords: MCFA, broiler, basal diet, performance, bacterial load

S1- 0498 Comparison between formulated and imported Vitamin Mineral Premix in commercial broiler chicken

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This study was conducted with commercial broiler chicken to compare the efficacy of formulated vitamin mineral premix (FVMP) with imported vitamin mineral premix (IVMP). A total of 270 day-old broiler chicks were randomly weighed and assigned to six dietary treatments (Control, FVMP1, FVMP2, FVMP3, IVMP1 and IVMP2) having 45 birds in each group following CRD experimental design. Each dietary treatment had three repetitions with 15 birds and was reared in floor pens. All the individual vitamin and minerals were purchased and vitamin mineral premixes (VMP) were formulated following the recommendations of BSTI, 2005 for broiler chicken as per requirement. The premixes were developed in three different concentrations (low, medium, high) of VMP. Body weight gain per unit of feed consumption was observed statistically similar in medium level of FVMP compared to IVMP of the diet. Feed conversion ratio (FCR) reduced significantly ($P < 0.05$) in different concentration of VMP in both formulated and imported VMP contained diet compared to control diet. Finally it could be concluded that the imported VMP may be replaced with low to medium concentration of IVMP in the diet for broiler production in Bangladesh.

Keywords: vitamin mineral premix, growth performance, feed conversion ratio, broiler chicken

S1- 0499 Performance indices of broiler chicken administered garlic (*A. sativum*) feed additive

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The feeding potentials of garlic (*A.sativum*) as feed additive in broiler diets was studied in 8 weeks. A total of 156 Marshall broiler birds were randomly allotted to 0g/kg (Control), 5g/kg, 10g/kg and 15g/kg levels of garlic treatments consisting of three replicates of 13 birds each in a Completely Randomized Design (CRD). The results obtained showed that significant differences ($P < 0.05$) existed in all the parameters measured vis-à-vis final weight, weight gain, feed intake and feed conversion ratio. However, results obtained revealed that using garlic as feed additive in broiler diets at 15g/kg depressed feed intake, improved growth rate, feed efficiency and reduced abdominal fat drastically. It was therefore concluded that the findings of this study will serve as useful information for feed resources and nutrition.

Keywords: abdominal fat, broiler chickens, feed intake, garlic, weight gain

S1- 0500 Effect of *Moringa oleifera* leaf powder supplementation on morphometric characteristics of tibia bone of broiler chicken

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Growth-promoting antibiotics (GPA), although beneficial for broiler chicken growth, are a source of public health hazard. Therefore, there is a pressing need for alternate sources which can act as growth promoter. *Moringa oleifera* is one such phytobiotic which is reported to possess antimicrobial and immuno-modulatory properties. This study aimed to investigate the effects of supplementation of *M. oleifera* leaf powder (MOLP) on morphometric characteristics of tibia bone in broiler. Day-old broiler chicks ($n=100$) were randomly divided into five groups with four replicates each ($n=5$). Birds were fed a corn-based basal diet or the same diet supplemented with 0.6, 0.9, 1.2 and 1.5% MOLP. On day 35, two birds per replicate were de-capitated to collect right and left tibiae of each bird as drumstick with flesh intact. The drumsticks were immersed in boiling water (100°C) for ten minutes and cooled at room temperature. Drumsticks were de-fleshed and air dried for 24 hours at room temperature. Data were analysed through one way analysis of variance and Duncan's multiple range test. All differences were considered significant at $P < 0.05$. The length, bone diameter, medullary canal diameter, Tibio-Tarsal Index of right and left tibiae bones did not vary ($P < 0.05$) among groups. The weight of right and left tibiae bone were significantly high ($P < 0.05$) in 1.2% MOLP group compared to control group. The ash content (%) was higher ($P < 0.05$) in all MOLP supplemented groups in right tibia but only in 0.6% MOLP supplemented group in left tibia compared to control group. The supplementation of 1.2% and 1.5% MOLP increased ($P < 0.05$) the weight/length index of the right and left tibiae compared to control group. The Robusticity Index was higher ($P < 0.05$) in control group as compared to all MOLP supplemented groups. It may be concluded that supplementation of MOLP partially improved tibial morphometric parameters with inclusion level of 1.2% being most effective.

Keywords: phytobiotics, tibia ash, robusticity index, tibia weight

S1-0501 Production performance of Pearl Grey guinea fowl laying hens fed diets varying in Lysine concentrations

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Guinea fowl production in the United States, Europe and other parts of the world is gaining momentum due to increase in demand of specialty poultry meat. However, optimal requirement for nutrients such as the amino acid lysine is lacking. Therefore, the objective of this study was to evaluate optimum levels of lysine needed for production performance of the Pearl Grey guinea fowl (PGGF) at 26-50 weeks of age (WOA). In a completely randomized design, 240 22-week old PGGF replacement pullets were assigned to dietary treatments comprising eight concentrations of digestible lysine (0.80, 0.86, 0.92, 0.98, 1.04, 1.10, 1.16, and 1.22%). Each of the diets contained 2,800 Kcal ME/kg of diet and 18% CP and was replicated 3 times with 10 birds per replicate. The diets were fed ad libitum for six 28-day periods from 22-50 WOA and experimental birds were raised in laying cages and received 16 hr light throughout the study period. The birds were observed for feed consumption (FC), hen-day egg production (HDEP), egg weight (EW), egg mass (EM), feed conversion ratio (FCR), internal egg quality (IEQ), shell thickness (ST) and body weights gain (BWG) at the end of each 28-day lay period. Birds fed the 0.92% lysine diets had higher percent HDEP, EW and EM, and lower FCR than those fed diets containing other lysine concentrations ($P < 0.05$). However, differences in FC, IEQ, ST and BWG among dietary lysine concentrations were not significant ($P > 0.05$). Therefore, since birds responded better to diets containing 0.92% digestible lysine, we recommend that PGGF layers be fed diets containing 0.92% digestible lysine from 26-50 WOA.

Keywords: pearl grey guinea fowl, egg production, digestible lysine, production performance

S1-0502 Evaluation of Effective Microorganisms on performance, carcass quality, gut microbial population and nutrient utilization in broiler chicken

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An experiment was undertaken to evaluate the effect of Anim Pro[®] Effective Microorganisms (EM) on the performance, meat quality, gut microbial population and nutrient utilization of broiler chicken. A total of 168 day-old Cobb 500 male broiler chicks housed in 12 deep litter pens were randomly assigned to 4 EM treatments with 3 replicates. EM was directly mixed with drinking water for broilers as 0.1 % EM (T1), 0.075% EM (T2) and 0.05 % EM (T3) while control group (T4) received drinking water without EM. On 36th day, 2 birds from each replicate were sacrificed to evaluate meat quality, gut microbial population, weight of lymphoid organs, dressing percentage, breast meat percentage and abdominal and subcutaneous fat percentage. Ileal content was collected to analyze the apparent ileal digestibility of nutrients. Broiler chicken treated with EM had a higher growth rate, improved feed efficiency and nutrient utilization ($P < 0.05$). The highest feed conversion ratio was observed in T4 (1.85) and the lowest was observed in T2 (1.54). The lowest dressing percentage (66.80%) and water holding capacity (50.65%) were observed in T4 ($P < 0.05$). Coliform counts in ileum in birds fed with T1 and T4 were 5.42 and 5.91 CFU/g, while in caecum the values were 6.19 and 6.48 CFU/g, respectively. The lowest crude protein (CP) and crude fat (CF) digestibility values were observed in T4 (70.63% and 69.23%, respectively) while the highest CP and CF digestibility values were observed in T3 (75.80% and 70.02%, respectively). Results from the current study revealed that EM was effective in improving the performances of broilers on tested parameters showing a greater potential of enhancing birds' health, decreasing mortality level and thereby reducing the need for antibiotics.

Keywords: Anim Pro[®], broilers, meat quality, nutrient utilization, gut microbial population

S1-0503 Variability in the fatty acid composition of excreta in broilers

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Manipulation of fatty acid content (e.g. saturates, n-7 and n-9 monounsaturates and n-3 and n-6 polyunsaturates), in chicken feed formulation by adding different types of fats is commonly used for economical, nutritional and other purposes. Previous studies have found correlations between the fatty acids content of diets and tissues that vary from strong, to weak or unrelated, depending on tissue type but it is not clear how the fatty acid profile of faeces correlates to diet. Studying this relationship could be important in aspects such as tracking dietary intake in birds allowed access to free-ranging environments, better understanding nutrient digestibility and metabolism efficiency, and changing manure composition. For 42 days, Cobb 500 male chickens (n=480) were fed one of 6 different diets supplemented with 4% (w/w) flaxseed (high n-3), corn (high n-6), macadamia (high n-7), canola (high n-9), coconut (high saturates) oils or beef tallow (control). Fresh wet faecal samples were collected for fatty acids analysis. No significant differences ($P \leq 0.05$) on growth rate or feed conversion ratio were observed between the different dietary treatments. Our results showed significant positive linear correlations ($R=0.89-0.99$; $P \leq 0.05$) between the feed and faecal fatty acid composition across all the treatments. An important finding was that at the dietary level of fat used in this trial, the birds (or their gut microbiota) appeared to preferentially utilise n-3, n-9, n-7 and saturated fatty acids, but not n-6 fatty acids even when they were most abundant. This suggests that oils which are high in n-6 fatty acids may not be ideal in broiler formulations. In general, the results suggest that faecal analysis can be a useful indicator of dietary fatty acid intake and utilization in broilers.

Keywords: fatty acids, broilers, diet, faeces

S1- 0505 Impact of mycotoxins deoxynivalenol and fumonisins on intestinal microbiota in broiler chickens

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Deoxynivalenol (DON) and fumonisins (FBs) are the most frequently encountered mycotoxins produced by *Fusarium* species. These two mycotoxins are ubiquitous in animal feed, contaminating more than 50% of cereal samples collected from European countries. Following ingestion of mycotoxin contaminated feed, the intestinal microbiota will be exposed to these toxins. The gut harbours a complex community of over trillions microbial cells which influence animal physiology, metabolism, nutrition and immune function, while disruption to the gut microbiota has been linked with diseases such as dysbacteriosis in poultry. Previously, we have demonstrated that the ingestion of FBs contaminated feed by broiler chickens for 15 days resulted in a reduced diversity of the ileal microbiota in broiler chickens compared to the control group receiving no FBs. Feeding a FBs contaminated diet to broiler chickens was correlated with a decrease in the abundance of the immunomodulating segmented filamentous bacteria (SFB) and *Lactobacillus johnsonii*. The aim of this study was to investigate further the impact of the mycotoxins DON and FBs on the development and composition of the intestinal microbiota in broiler chickens. One-day-old broiler chicks (Ross 308) were divided into three groups, each consisting of three pens of 30 birds each, and were fed either a control diet, a DON contaminated diet or a FBs contaminated diet. The contamination levels were approaching the maximum European guidance levels for poultry feed (2006/576/EC). Subsequently, after a feeding period of one, three and five weeks chickens were euthanized and samples of intestinal digesta and mucus were collected from different intestinal segments. The impact of mycotoxin exposure on the luminal and mucus associated intestinal microbiota was investigated based on 16S rRNA sequencing and qPCR. Results will be presented at the conference.

Keywords: broilers, deoxynivalenol, fumonisins, microbiota, mycotoxins

S1-0506 Effect of commercial poultry feed enzyme preparation on degradation of non-starch polysaccharides and phytate phosphorus in sunflower oil cake by in vitro for poultry feed preparation

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An experiment was conducted to study the effect of commercial poultry feed enzyme preparation on degradation of non-starch polysaccharides (NSPs) and phytate phosphorus in sunflower oil cake (SFOC) by in vitro. The feed enzyme preparations used in this study were Cellulase 146 IU/g, Xylanase 241 IU/g, Pectinase 98 IU/g, Protease 74 IU/g, Amylase 778 IU/g and Phytase 33 IU/g. Four levels of feed enzymes at 0, 2.5, 5.0, 10.0, 15.0 mg/10g of SFOC was taken in 100 ml beaker with 40 ml of 0.2 M acetate buffer and was incubated at 42°C for 2 hours simulating the poultry gut system. After the incubation the contents were mixed well and allowed to stand for 10 minutes, then they were centrifuged and the supernatant was used for glucose, reducing sugars and inorganic phosphorus estimation. The linear increase in the amount of glucose released from SFOC viz. 4.91, 6.69, 12.07 and 15.03 % in 2.5, 5.0, 10.0, 15.0 mg of enzyme/10g of SFOC respectively over control. The amount of reducing sugar was 1365.62 mg % in control which was increased by 2.45, 4.44, 5.67 and 7.08 mg % by addition of the commercial feed enzyme mixture at 2.5, 5.0, 10.0 and 15 mg/10 g of SFOC respectively. The amount of inorganic phosphorus (%) released in the control was 0.052 % which increased to 0.056, 0.066, 0.074 and 0.082 % by addition of 2.5, 5.0, 10.0, 15.0 mg of commercial feed enzyme preparations per 10 g of SFOC respectively and increase over the control was by 7.14, 21.21, 29.73 and 36.59 % respectively. It was concluded that the commercial poultry feed enzyme preparation was increased the availability of non-starch polysaccharides and phytate phosphorus through increased release of glucose, reducing sugars and inorganic phosphorus from SFOC. This was useful indicator to identify the quality of crude commercial enzyme preparations used for poultry ration and also, it might be very helpful when formulating the ration contains feed ingredients with high levels of NSPs and phytate phosphorus

Keywords: non starch polysaccharides degrading enzymes, phytase, sunflower oil cake

S1-0507 Investigation of duck chicks feeding by sea coast as a complete ration

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An experiment was conducted in order to evaluation of nutritional potential of Caspian Sea's Coast on feeding of duck chicks. A completely randomized design with six treatments group, four replicates per treatment and totally 480 Pekin duck chicks were used. Duck chicks reared in ordinary pens until 14 days old and then transferred into near coastal water for 42 days. Treatments included: A) Feeding up to total requirement (NRC, 1994) without access to coastal water, B) Feeding up to total requirement with access to coastal water, C) Restricted feeding up to 75 % of total requirements with access to coastal water, D) Restricted feeding up to 50 % of total requirements with access to coastal water, E) Restricted feeding up to 25 % of total requirements with access to coastal water, and F) No manual feeding and only access to coastal water. The result showed that there are significant differences in final body weight and daily weight gain between treatments, so that, treatments B and F had highest and lowest amounts of this parameters respectively ($P < 0.05$). Feed conversion ratio increased in treatment A, but decreased significantly in treatment F ($P < 0.05$). Treatment B feeding strategy leading to highest carcass weight, carcass weight to live weight ratio, leg and Breast relative weight ($P < 0.05$). In conclusion, restricted feeding of duck chicks up to 50-75 % of requirement with open access to coastal water may be appropriate.

Keywords: duck chicks, Coastal water, Restricted feeding

S1-0508 Gross performance of broiler chickens on different diet forms containing varying levels of whole sorghum grain

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The interest in use of whole-sorghum grain in chicken diets is increasing and is mainly driven by economic factors. There are inconsistencies in optimum inclusion level of whole grain, type and quality of grain, age of birds, and feeding regime. Reports on the use of sorghum whole grain in broiler diets are limited. Therefore, the purpose of this study was to determine the effects of feed form and different whole sorghum grain inclusion rates on the productivity and digestive organ development of broiler chickens. Four diets containing 100 % rolled sorghum or 25, 50 and 75 % whole sorghum replacing rolled sorghum were formulated and fed as either mash or pellets. The eight diets were fed to a total of 432 day-old broiler chickens from hatch to 35 days of age. Body weight and feed intake were measured on a pen basis at 10, 25, and 35 days of age and feed conversion ratio (FCR) calculated. The general linear model procedure of the statistical analysis software (SAS) was used to analyse the data. Feed form had an influence ($P < 0.05$) on bird performance from 1-35 days of age. Pellet diets improved ($P < 0.05$) feed intake, body weight and carcass parts of broiler chickens aged 1-35 days. A treatment interaction for body weights at ages 1-24 ($P < 0.0021$) and 1-35 ($P < 0.0019$) days was observed. The relative weight of the gizzard was increased and gizzard pH was reduced ($P < 0.05$) at 35 days of age for birds offered mash diets. Feed conversion ratio (1-35 days) increased ($P < 0.035$, quadratic effect) with an increase in whole sorghum and levelled off with higher inclusion rates. The relative gizzard weight at 35 days was marginally increased ($P < 0.033$, linear effect) with increase in whole sorghum inclusion levels. Overall, the results showed that pelleted diets were superior to mash diets. Although higher levels of whole sorghum inclusions enhanced the gizzard development, performance parameters of birds offered these levels were not affected.

Keywords: Pellet, mash, gizzard, pH, meat yield

S1-0509 Influence garlic extract in diet on performance and health of broiler in Indonesia

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Tropical climate can have a damaging effect on performance and health of broiler in Indonesia. The infections are strongly affected by climatic condition such as temperature, rainfall, and humidity. The research was conducted to evaluate the effects of supplemented garlic extract (GE) in diet on performance and health of broiler in Indonesia. Ninety-six day old chicks were equally divided into four groups, three replications and eight chicks each. Group 1 was control, then GE was supplemented to the experimental group 2, 3 and 4 (2, 4, 6% in diet). The research was conducted for five weeks period from day old chick to 35 days. Feed and water were offered ad libitum. Body weight of chicks were determined at day 1 and 35, feed intake was determined at the same period, feed conversion ratio was calculated accordingly. At 35 day age, four chicks from each replicate were randomly selected and weighed to obtain body weight. Two ml of blood were collected from each sample. The blood was discharged immediately into collection tube with anticoagulant for examination of the differential count of total white blood cell. Data were analyzed using repeated measurements of the ANOVA and Duncan's Test was used to determine of significance between means. Diet with GE (2, 4, and 6%) significantly ($P \leq 0.05$) increased the body weight, gain weight, and decreased feed conversion. Feed consumption showed no significant difference ($P \geq 0.05$). Chicks treated with different level GE in diet showed significant increase in serum total protein, albumin, A/G ratio, and immunoglobulin G compared to control. These results showed the beneficial effect of GE to improve immunity status. There were no significant difference ($P \geq 0.05$) on differential count of white blood cell including neutrophil, eosinophil, monocytes, and lymphocytes between the different treatments. It is concluded that supplementation GE in ration significantly enhanced health of broiler without any adverse effects.

Keywords: garlic extract, performance, immune, health, broiler

S1-0510 Investigation in supplementation of diets with some oil sources on broiler performance

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Two experiments were carried out to determine the AME content of four dietary fat sources including refined soybean oil (SBO), hydrogenated soybean oil (HSBO), recycled soybean oil (RSO), and acidulated soybean oil soap stocks (ASO), and to investigate the effects of the use of these oils in diets (3.5%) on growth performance of broilers from 1 to 42 days of age. In the first experiment, the AME of the oil sources were determined in Leghorn roosters (n=5) fed diets containing 5% of them, through either multiplying the digestibility of the oil by its gross energy value or difference between the AME values of the basal diet and a diet containing 5% of the oil. In the second experiment, a Complete Randomized Design was employed using four treatments replicated five times and twelve broiler chicks in each. The AME of the oils obtained by multiplying their GE by the corrected TTAD coefficient of the added fat appeared to be highest for the SBO (8,920 kcal/kg), HSBO (8,733 kcal/kg) and RSBO (8,602 kcal/kg) and lowest (7,836 kcal/kg) for the ASBO. When calculated by difference, the AME of the oils Turned out to be 9,016, 8,794, 8765 and 7,906 kcal/kg for SBO, HSBO, RSBO and ASBO, respectively. The results of second experiment indicate that supplementation of different sources of oils have no effect of growth performance of broilers during 42 days. It is concluded that when conveniently processed, recycled soy oil, hydrogenated soy oil and acidulated soy oil soap stocks can be regarded as good substitutes for refined soybean oil in diets for broilers.

Keywords: broiler performance, ame, fat and oils

S1-0512 Effect of in ovo feeding on the production performance and intestinal histomorphometry of commercial broilers

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A nutritional feeding trial was carried out to investigate the effect of in ovo feeding of various nutrient solutions on the growth performance and gut development in commercial broilers (Cobb-400). A total of 540 fertile eggs with uniform weight were randomly divided into six groups each with three replicates of 30 eggs each. In ovo injection was carried out on 18th day of incubation with one of the following nutrient solutions 0.5 ml of 10 % glucose (T3), 0.5 ml of 0.5 % lysine (T4), 0.5 ml of 0.5 % threonine (T5), 0.5 ml of 0.5 % β -hydroxy- β -methylbutyrate (HMB) (T6) into the amnion along with non injected (T1) and injected (0.5 ml of normal saline solution) (T2) control. 288 chicks were randomly allotted into 6 treatments each with 3 replicates of 16 chicks each. The birds were provided with ad libitum feed (BIS 2007) and water. The tissue samples (2 cms) were taken from duodenum, jejunum and ileum for histomorphometric measurements. Data recorded in the biological experiments were subjected to one way analysis of variance (ANOVA). Results revealed that the in ovo feeding significantly ($P < 0.05$) improved hatchability and transit weight loss was significantly ($P < 0.05$) reduced. In ovo feeding with lysine and threonine produced significantly ($P < 0.05$) heavier day old chicks. Numerically higher body weight was observed in threonine fed group (2195.86 g), while the control birds had the lowest body weight of 1211.74. The mean cumulative feed consumption was significantly ($P < 0.01$) low in threonine, HMB and lysine injected chicks compared to other groups. FCR was improved significantly ($P < 0.05$) in threonine and HMB groups than control. In ovo feeding of significantly ($P < 0.05$) improved duodenal, jejunal and ileal histomorphometry compared to control. From this study it was concluded that in ovo feeding has improved the growth performance and gut development of broilers which resulted in more production performance.

Keywords: In ovo feeding, broilers, growth performance, gut histomorphometry

S1- 0513 Effect of commercial feed enzyme supplementation on intestinal viscosity of broilers on nutrient reduced diet

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An experiment was conducted to assess the effect of commercial feed enzyme supplementation on intestinal viscosity of broilers. Broilers were fed with enzyme at 0, 250, 500, 750 and 1000 g/ ton of feed with a proportionate reduction in metabolizable energy (ME- 0,1.25, 2.5, 3.75 and 5 %), crude protein (CP- 0,0.75, 1.5, 2.25 and 3 %), methionine+cystine (0,0.5, 1, 1.5 and 2 %) and available phosphorus (AP-0 2.2, 4.4, 6.6 and 8.8 %). Enzyme used in this study contained Cellulase 146 IU/g, Xylanase 241 IU/g, Pectinase 98 IU/g, Protease 74 IU/g, Amylase 778 IU/g and Phytase 33 IU/g. One hundred and sixty five Ven Cobb broiler chicks were weighed individually and distributed randomly to five experimental diets with three replicates of eleven chicks each. At the end of six weeks of age, six birds from each experimental group were selected randomly and slaughtered for estimating digesta viscosity. The intestinal viscosity in the birds fed with diet containing enzyme at 0, 250, 500, 750 and 1000 g/ton of feed was 2.26, 1.85, 1.67, 2.01 and 2.00×10^{-3} NSm-2 respectively. A significant ($P < 0.05$) decrease was observed in the intestinal viscosity by 18.14% and 26.11% in 250 and 500 g/ton of enzyme supplementation than control. There was no significant difference in viscosity among 0, 750 and 1000 g enzyme supplemented groups. The dry matter (DM) content of intestinal digesta was also significantly ($P < 0.05$) increased in all the enzyme supplemented groups than control. It was concluded that the commercial feed enzyme supplementation up to 500g enzyme per ton of feed was significantly reduced the viscosity of the intestinal digesta of broilers. This might be improves the utilization of non-starch polysaccharides by feed enzymes in broilers.

Keywords: feed enzymes, broiler, intestinal viscosity

S1- 0514 Zearalenone degradation by probiotics bacillus amyloliquefaciens form herbivores feces

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Zearalenone(ZEN) is a mycotoxin produced by a number of species of Fusarium which impose threat to humans and animals health, ranging from the reproductive toxicity, cytotoxicity, genetic toxicity and immune toxicity. The aim of this study was to screen microbes from herbivores feces for their zearalenone degrading potential and select probiotics whose activities do not create toxic metabolites. Forty strains were screened out on the selective medium which the ZEN was the sole carbon nutrient in our preliminary experiment. As a results of zearalenone biodegradation experiments confirmed by ELISA that the H6 which was isolated from rabbit feces present the highest degradation rate up to 85%-93% when 100μL ZEN(1μg/mL) incubated with 900μL culture supernatant for 72 h at 37°C. The strain H6 was identified as Bacillus amyloliquefaciens by 16S rRNA sequence analysis. Our findings demonstrate that the degradation activity mainly distribute in the fermentation broth rather than the cell extracts. The degradation ability was not reduce treatment with proteinase K, proteinase K plus SDS or heating. These results suggest that the strain H6 is a potential probiotics for degrading ZEN and can provide a new approach for the detoxification of ZEN.

Keywords: Zearalenone, biodegradation, probiotics, Bacillus amyloliquefaciens

S1-0515 The effect of in ovo supplementation of arginine and/or tryptophan on hatchability, immune responses and muscle diameter of broiler chicken

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To evaluate hatchability, immunity and muscle diameter of broiler in ovo fed (IOF) with arginine (Arg) and tryptophan (Trp) into amnion through broad end of egg of 34wk old broiler breeder using 25mm needle. Total 700 fertile eggs with uniform egg weight were collected and divided into five treatment groups: non-injected control, injected control (0.5ml of 0.9% normal saline), arginine (0.5ml of 0.5% Arg), tryptophan (0.5ml of 0.5% Trp) and combination (0.25ml of 0.5% each of Arg and Trp) and injected on 18d incubation. On 21d, 108 chicks were randomly selected from each treatment and divided into six replicates of 18 in each. Spleenocytes were collected on 4, 17 and 32d to study expression of IFN- γ and IL-8 using qPCR. Muscle diameter was studied with the help of a light microscope equipped with calibrated micrometer. Analysis of data: ANOVA, SPSS V.20 and χ^2 test (hatchability). Hatchability in Arg and Trp injected groups were greater than un-injected control group. Arg injected eggs had higher chick weight ($P<0.01$) and chick/egg weight ratio compared with untreated control group. Expression of IFN- γ gene was significantly higher in IOF Arg and Trp group than all other treatment groups. IL-8 did not express itself in all amino acid treatment groups. HI titer against ND virus on 4d was more in all treatment groups than control. Throughout study (4, 21 and 35d), birds fed with Trp exhibited significantly higher ($P<0.01$) breast muscle diameter. On 4 and 35d alone Arg fed group exhibited comparable breast muscle diameter to Trp and combination group. Arg and Trp are critical for growth of chicken embryo; however, during post-hatch period Arg played more significant role. In ovo injection of Arg and Trp acts as immunomodulator and enhance muscle diameter.

Keywords: broiler production, in ovo feeding, arginine, tryptophan and combination (arginine and tryptophan)

S1- 0516 Determination of non-starch polysaccharides and true metabolizable energy content in several wheat varieties

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The aim of this experiment was to determine non-starch polysaccharides and true metabolizable energy of five wheat varieties (Alvand, Zarrin, Sabalan, Sardari and Azar2). Non-starch polysaccharides (NSP) of wheat varieties were measured with Englyst method by spectrophotometer. Nitrogen corrected true metabolizable energy of five wheat varieties was also determined with Sibbald precision feeding method. A completely randomized design with 24 roosters (Ross 308) at the age of 5 month was used in this experiment. The result of NSP content showed the total NSP, Insoluble NSP and soluble NSP varied of 9.44 to 11.96 %, 7.10 to 9.40 % and 1.95 to 3.15 % DM, respectively. The highest total NSP value (11.96%) and Insoluble NSP value (9.40%) were found in Aza2 variety, and the highest Soluble NSP value (3.15%) was found in Alvand one. The lowest total NSP (9.44%) and soluble NSP (1.95%) values were resulted of Sabalan variety, and the lowest Insoluble NSP (6.94%) was resulted of Zarrin variety. The TMEn content of wheat varieties varied from 3562.32 Kcal/kg (Sabalan variety) to 3741.64 Kcal/kg (Zarrin variety), and TMEn content showed significant differences as wheat varieties ($P<0.01$). This experiment demonstrated that the metabolizable energy content of East Azerbaijan wheat varieties have a high variation and relative to NSP content of wheat varieties.

Keywords: true metabolizable energy, non-starch polysaccharides, wheat varieties.

S1-0517 Effect of herbal methionine (Methiorep) on the production performance of layers

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A nutritional feeding trial was conducted to investigate the effect of herbal methionine (Methiorep) on the production performance of layers. 225 Rhodo White layers were assigned randomly in to five treatment groups each with three replicates of 15 layers and fed with one of the following experimental diets viz., Control(T1), basal diet was supplemented with DL Methionine@2 Kg/tonne of feed (T2), DL Methionine1.33Kg + Methiorep@0.67Kg/tonne of feed (T3), DL- Methionine1Kg + Methiorep1Kg/tonne of feed (T4), Synthetic DL Methionine 0.67 Kg + Methiorep@1.33 Kg/tonne of feed (T5). The experiment was carried out for 20 weeks. The birds were provided with standard layer ration (as per BIS 2007) and water ad libitum. Data recorded in the biological experiment were subjected to one way analysis of variance (ANOVA). Results revealed that there was significant improvement in hen day and hen housed egg production in 50 and 66 % replacement of synthetic methionine with Methiorep. The peak production was long with slow tapering in Methiorep supplemented groups. FCR was noticed in all Methiorep supplemented groups. Marginal improvement in external egg quality was observed in Methiorep supplemented group compared to control. Non significant improvement in the internal egg quality characters was found in Methiorep supplemented groups. Optimum shell thickness was noticed in methionine supplemented groups irrespective of the source compared to groups fed with methionine deficient diet. The livability was better in Methiorep supplemented groups. Herbal methionine (Methiorep) at the rate of 33, 50 and 66 per cent reduced pullet production cost of Rs.8.86, 10.98 and 14.23 respectively. Methiorep at the rate of 33, 50 and 66 per cent inclusion, the egg production cost was reduced to the tune of Rs.0.07, 0.17 and 0.27. Methiorep supplementation has positive effect on the production performance of layers.

Keywords: layers, herbal methionine, egg production, serum biochemistry

S1-0519 The effects of different levels of wet extruded full fat soybean (ShaySoy™) in reproduction traits of layer breeders

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The effects of different levels of extruded full fat soybean (ShaySoy™) in reproduction traits, immune responses of layer breeders were assessed in this research. For this purpose 108 layer breeder hens) White Bovans(and 12 males were allocated on a randomized complete block design with three treatments and four replicates in litter pens with 10 birds in each, for a 60 days experimental period. Treatments included 1. Control diet based on corn/soybean meal and oil 2. Diet containing 7.5% extruded full fat soybeans 3. Diet containing 15% extruded full fat soybeans. The effects of treatments on layer breeder performance, reproduction traits and hatchability were determined. For evaluating the immune response, haemagglutination inhibition (HI) titers against Newcastle disease and immune response against sheep red blood cell (SRBC) for humoral immunity and swelling of the membrane between the toes after injection of phytohaemagglutinin (PHA-P) as an indicator for cellular immunity was considered. Results showed that inclusion of FFSB in diet had no effect on egg production but by increasing FFSB level, the average egg weight increased significantly ($P < 0.05$). The fertility and hatchability were affected by treatments ($P < 0.05$). By increasing FFSB level the male and female chick weight were increased by increasing FFSB level ($P < 0.05$). Antibody against NDV by HI method were affected by different treatments in days 42 and 56 of experiment ($P < 0.05$). SRBC test at 33 and 38 days of experiment were not affected by different treatments ($P > 0.05$). Although swelling of the toes membrane after PHA-P injection at 35 days of experiment increased in EFFSB7.5 ($P < 0.05$) compared to other treatments, but this reaction were not affected by any treatments at 55 days of experiment ($P > 0.05$). In conclusion, based on the results of this research, that inclusion of EFFSB in level of 7.5 and 15 percent of layer breeder diet had positive effects in reproduction traits, and immune response.

Keywords: extruded full fat soybean, hatchery, immunology, hatchery

S1-0520 Study of different levels of mannan oligosaccharide prebiotic (ActiveMos™) on performance and immune responses of broiler chickens

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This project was conducted to investigate the effects of different levels of mannan oligosaccharide prebiotic (ActiveMos™) on performance and immune responses of broiler chickens. For this purpose, 500 day old broiler chicks (Ross® 308) were used. These chicks were allocated by a completely randomized design in five treatments, including four replicates that in each 25 broilers were kept for 42 days in litter pens. Treatments included a control group that fed a common broiler diet without any feed additive. Positive control group received control diet plus Avilamycin (0.1%), Prebiotics groups, received control diet plus 0.05%, 0.1% and 0.2% levels of mannan oligosaccharide prebiotic (ActiveMos™). Antibody titers against Sheep Red Blood Cell (SRBC) were measured at days 33 and 38 of the experiment, the response to injection Phytohaemagglutinin (PHA) at day 36 and antibody levels against Newcastle disease at days 14, 28 and 42 were evaluated. At the end of the experimental period from each pen two broiler chickens were slaughtered and carcass yield and organ weight were measured. Results showed that in this research, performance were not affected by prebiotic levels in the feed. Also there were no significant differences in carcass yield among experimental treatments and control group. The prebiotic levels had no significant effect on SRBC titer. Significant increase ($P \leq 0.05$) in cellular immunity was found after PHA injection in positive control and prebiotic experimental groups compare to control treatment and the 0.2% prebiotic treatment had the most increase in cellular immune response. The comparison serum Haemoagglutination Inhibition (HI) titer against Newcastle disease showed that prebiotic levels had significant differences at day 42 in prebiotic treatments. Finally from this study, we concluded that the use of prebiotic have positive effects on some immune responses in broiler chickens.

Keywords: broiler, mannan oligosaccharides, performance, SRBC, PHA, HI

S1-0521 L- methionine incorporation into the breast muscle of broiler chickens at the initial fase

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The commercial availability of stable isotope labeled compounds associated to highly precise mass spectrometers has made possible to provide accurate metabolite identification and investigation. This research highlights aspects of broiler nutrition and metabolism in which [methyl- ¹³C] substrates can be applied to investigations of amino acid dynamics in animal organisms. The objective of the study was to assess the rate of labeled methionine incorporation into the breast muscle of broilers aged 1-7 d-old. A total of 51 one-d-old male Cobb broiler chickens, with an initial BW of 45 ± 1.13 g, were housed (12 birds/m² density) after selection by weight. The diet was formulated based on corn and soybean meal. A dosage of 29 μ mol of L-[¹³C]methionine/kg BW/h was used, administered orally within 6 h (99 atom% ¹³C, Cambridge Isotope Laboratories, Inc.) to promote tissue enrichment. At 0 (control), 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 48, 72 and 96 h after initial dosing, were slaughtered 3 birds per time-point, and collected breast muscle samples, which have undergone lyophilization and milling processes. Carbon isotopic analysis was obtained using a mass spectrometer. Second-order polynomial fit was used to determine the maximum incorporation point, and analyzed by first-order exponential equation $[\delta^{13}C(t) = \delta^{13}C(f) + [\delta^{13}C(i) - \delta^{13}C(f)]e^{-kt}]$, obtained using the statistic software Minitab® 16. Maximum enrichment occurred 12.4 h after oral administration of enriched solution, and resulted in the equation: $\delta^{13}C = -17.50 - 1.58e^{-0.2532t}$ ($r^2 = 0.98$), with half-life $[T = \ln 2/k]$ of 2.7 h, representing the velocity of methionine incorporation into embedded tissue. Thus, the time required for 50% of orally-administered labeled methionine to be metabolized is approximately 2.7 h at this dose and age of bird.

Keywords: pectoralis major, traceability, turnover

S1- 0523 Comparative effect of different proteases in nutritionally marginal diet on the performance and nutrient utilization of the broilers

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This study was conducted on male broiler chickens (Hubbard) to investigate the comparative effect of different commercially available proteases in the broiler diet. Chicks were randomly divided into 5 treatment groups having 5 replicate of 25 chicks each. Five isocaloric (ME: 2825 kcal/kg) diets were formulated and designated as positive control (PC; CP 19.5%), Negative control (NC; CP 19%), Cibenza (NC+Cibenza DP 100 @ 0.05%; CB), Ronozyme (NC+Ronozyme @ 0.02%; RZ) and Winzyme (NC+Winzyme plus @ 0.02%; WZ). The NC diet contained 7% less digestible amino acids as compared with PC diet. Growth performance, carcass characteristics, dry matter digestibility and nitrogen retention were investigated. Live body weights of broiler fed the PC diet were significantly higher ($P < 0.05$) than those fed NC diet. Birds fed CB, RZ and WZ diets grew as well as the broilers fed the PC diet. Feed intake of the broilers fed PC diet was higher ($P < 0.05$) than the broilers of NC group. There was no difference in the feed intake of broiler fed diets supplemented different proteases compared to NC. Mortality corrected feed conversion ratio (FCR) was impaired in birds fed the NC diet, but it was similar between those fed PC or different proteases (CB, RZ, WZ) supplemented diets. Different dietary treatments had not significant ($P > 0.05$) effect on the dressing percentage, thigh weight, heart weight, gizzard weight and abdominal fat. Liver weight percentage was higher for the broilers fed the NC diet and it was lowest for the WZ group. Dry matter digestibility was higher ($P > 0.05$) in RZ supplemented group and was lowest for the birds in NC group. Nitrogen retention was improved ($P > 0.05$) in the enzyme supplemented groups. In conclusion, enzyme supplementation improves the weight gain and feed conversion ratio, dry matter digestibility and nitrogen retention in low protein diets but there was non-significant differences of FCR among the PC and protease supplemented groups.

Keywords: broiler, proteases, growth performance, carcass characteristics, digestibility

S1- 0524 Humic acid improves growth performance in Japanese quail

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Use of antibiotic growth promoters has greatly been criticized in poultry due to issues of bacterial resistance and possible residual contents in meat and eggs. Meanwhile demand for organic and natural growth promoters is on the rise and efforts are going on to find suitable products. In this regard Prebiotics, probiotics and organic compounds have got great attention over the years. Humic acid is one of the organic compounds that is produced by degradation of organic matter and is a bio-stimulant derived from compressed carbon based materials. It is reported to have antibacterial, antiviral, anti-inflammatory properties, so may also be used as growth promoter. This study was carried out to investigate the effect of Humic acid on Japanese quail growth performance from 7 to 28 days of age. Two hundred and forty mixed sex Japanese quail were used in this study and they were subjected to four dietary treatments i.e. i) T1 = basal diet (negative control), ii) T2 = 125 gm Neomycin/50 kg feed as antibiotic growth promoter (positive control), iii) T3 = 75 gm Humic acid per 50 kg feed, iv) T4 = 150 gm Humic acid per 50 kg feed. Each treatment was offered to 60 quails in three replicates. The data on weekly body weight, feed intake and mortality were recorded and at day 28 birds were slaughtered to get dressing percentage and bone to meat ratio. The final body weight (105.4 ± 13) and feed conversion ratio (2.84 ± 0.5) were significantly ($P < 0.05$) lower in quails fed on T1 (negative control) diet compared with all other treatments. However, body weight ($T2 = 121 \pm 11$, $T3 = 118 \pm 11$, $T4 = 120 \pm 11$) and FCR ($T2 = 2.49$, $T3 = 2.51$, $T4 = 2.48$) did not vary between quails fed on either Humic acid or Neomycin diets. Furthermore, mortality, dressing percentage and bone to meat ratio did not differ ($P < 0.05$) between the treatments. We concluded that supplementation of diet with Humic acid increase body weight gain and feed conversion ratio in Japanese quail.

Keywords: organic substance, growth promoters, natural feed additives, broiler quails

S1-0525 Feed formulation on digestible amino acid basis increases cottonseed meal inclusion level in chicken broiler diet

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The proportion of indigestible amino acids varies between feed ingredients, so it is recommended that feed formulation should be done on digestible amino acid rather than total amino acid basis. Cottonseed meal (CSM) makes an economical source of protein for poultry feed however, its inclusion rate in the chicken broiler diet is limited due to poor digestibility of amino acids and presence of toxins. This study was carried out to evaluate the efficacy of corn based diets containing 10 or 15 % CSM added either on total amino acid or digestible amino acid basis, on broiler performance from day 21 to 35 of age. Two hundred and forty Hubbard broiler chicks were fed on four dietary treatments i.e. i) corn based diet containing 10 % CSM on total amino acid basis, ii) corn based diet containing 10 % CSM on digestible acid basis, iii) corn based diet containing 15 % CSM on total amino acid basis, iv) corn based diet containing 15 % CSM on digestible amino acid basis. Each treatment was offered to 60 broilers in three replicates. Data on weekly body weight gain and feed conversion ratio, and dressing percentage were recorded. The broilers fed on diets containing either 10 or 15 % CSM on digestible amino acid basis showed no significant difference in body weight gain (1707 ± 56 gm in 10 % CSM diet versus 1698 ± 61 gm in 15 % CSM diet) and feed conversion ratio (1.74 in 10 % CSM diet versus 1.76 in 15 % CSM diet), however these values were significantly ($P < 0.05$) better than broilers fed on diets containing either 10 or 15 % CSM on total amino acid basis. Nevertheless, dressing percentage did not vary ($P < 0.05$) between the treatments. We concluded that the performance of broilers fed on lower levels of cotton seed meal depend on availability of digestible amino acids rather than its inclusion level to the feed.

Keywords: broiler performance, cottonseed meal, feed formulation, digestible amino acid

S1- 0526 Effect of xylanase supplementation with and without added fat in broiler diets

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A trial was conducted to evaluate the effect of xylanase supplementation with and without added fat in broiler diets. In the trial 300 day old broiler chicks were used which were randomly divided into 30 replicates in a 3×2 factorial arrangement under Completely Randomized Design (CRD). Experimental diets were formulated using 0, 1 and 2 % fat with or without 100 mg/Kg of xylanase. Experiment lasted for 35 days. The results showed that fat and xylanase affected ($P < 0.05$) starter and overall feed intake but did not affect ($P > 0.05$) finisher feed intake. Xylanase supplementation affected ($P < 0.05$) starter feed intake however fat did not affect ($P < 0.05$) starter, finisher and overall feed intake. There was no effect ($P > 0.05$) of any combination of fat and xylanase on starter and final live weight but finisher weight gain was affected ($P < 0.05$). Addition of xylanase affected ($P < 0.05$) while fat did not affect ($P > 0.05$) starter, finisher and final weight gain. Overall FCR was affected ($P < 0.05$) by xylanase and combination of fat and xylanase but fat supplementation did not affect starter, finisher and overall FCR ($P > 0.05$). Fat supplementation did not affect ($P > 0.05$) dry matter and ether extract digestibility but combination of fat and xylanase improved it ($P < 0.05$). Carcass traits, organ weight and mortality were not affected by any treatment. It can be concluded from the trial results that xylanase supplementation in diets containing added fat might improve nutrient digestibility and weight gain in broilers.

Keywords: xylanase, fat, broiler

S1-0527 Glycerin as a source of energy in Japanese quail (*Coturnix coturnix japonica*)

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The objective of this study was to evaluate the effect of dietary inclusion of glycerin on growth performance feed intake, feed conversion ratio, blood cholesterol and carcass characteristics in Japanese quails. A total of 375 day old Japanese quails were randomly divided into five equal groups under Complete Randomized Design (CRD). Five iso-caloric and iso-nitrogenous diets were formulated. Control diet (without glycerin addition) and other with 2.5%, 5%, 7.5%, and 10% glycerin addition. Each treatment replicated 5 times and each replicate was containing 15 birds. Birds were housed in galvanized wire cages with flat deck having 15 birds in each cage, in a well ventilated shed. Fresh clean water and feed was available at ad-libitum basis round the clock. Feed intake and body weight was recorded on daily basis then calculated weekly basis from which feed conversion ratio was derived. At the end of experiment two birds (one male and one female) from each replicate were selected randomly and slaughtered to determined carcass characteristics. Data collected were subjected to statistical analysis under CRD. Feed intake and body weight gain was not affected ($P > 0.05$) with glycerin supplementation. Lowest feed intake was observed in birds fed diet with 2.5 % and highest weight gain was observed in birds fed diets with 7.5 % glycerin. Glycerin had significant effect on feed conversion ratio. The best feed conversion values ($P < 0.05$) were observed in birds fed diet supplemented with 2.5% glycerin. There was no effect of glycerin inclusion on blood cholesterol level of Japanese quail ($P < 0.05$). Glycerin supplemented diets did not affect on breast yield, leg quarter yield, carcass weight, liver, heart, gizzard, bursa, spleen, intestinal weight, and intestinal length quails. Therefore, it can be concluded that supplementation of glycerin at the level of 2.5 and 5% in the diet of Japanese quail had beneficial effect on growth performance.

Keywords: glycerin, energy source, japanese quails

S1-0528 Effect of different levels of dietary calcium sources (sea shells, calcium carbonate and limestone) on egg shells of commercial laying hens

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In this trial the effect of different levels of dietary calcium sources (sea shells, calcium carbonate and limestone) on egg shells quality characteristics of commercial laying hens (shaver strains) were study. 280 hens at the age of 57 wks in a completely randomized design with 7 treatments, 4 replicates and 10 birds each were used in three periods of 28 days. The treatments were: 1. The samples with 100% shellfish powder, 2. samples with 100% calcium carbonate, 3. samples with 100% limestone, 4. samples with 50% powdered shellfish and 50 percent of limestone, 5. samples, with 50% limestone and 50% calcium carbonate, 6. samples with 50% shellfish and 50% calcium carbonate, 7. samples with 33% calcium carbonate, 33% shellfish and 33% limestone. Statistical data showed that the supply of shellfish and calcium carbonate and limestone caused the highest weight, strength, egg shell thickness and density significantly compared with other resources ($p \leq 0.05$). The result of this experiment indicate that combination of three sources of calcium in the diet improves egg shell quality and the percentage of healthy egg production in commercial farms.

Keywords: calcium carbonate, egg quality, limestone, shellfish

S1- 0529 Poultry diseases related to feed quality in Ghana

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This paper seeks to find ways of how to improve the feed quality production in order to improve performance of the poultry farm to avoid diseases caused by pathogen related poultry feed in Ghana as poultry feed has formed about 60 – 70 percent of the total cost of production. Poultry feed production standards are very low, and are faced with disease challenges caused by feed contamination. This affect small- scale poultry producers especially rural poultry since most of the farmers depend on the production for their livelihood. There is therefore the need to pay attention to diseases related to feed quality, importance of feed and quality control management of day to day activities in order to prevent disease outbreak due to Salmonella. Awareness created among poultry farmers and feed producers through education. Building storage to solve the shortage of feed ingredient and protection of its quality to safe cost, education and training farmers to be aware and conversant with husbandry practices were documented, As well as drawing the attention of poultry farmers to be more concerns about feeding management practice to improve the quality of their operations. Expected outcomes will be that quality and safety production of poultry feed to prevent related diseases thus providing employment opportunity for the youths; stimulate and activate more opportunity for prospective Entrepreneurs.

Keywords: feed quality, diseases, contamination, safety and quality control

S1- 0530 Evaluation dietary hempseed and hempseed oil on performance, egg quality and some blood parameters in laying hen after peak period

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The aim of this study was to recognize the effects of dietary hempseed(HS) and hempseed oil (HO) on performance, egg quality and blood parameters of laying hens. A total of 320 Hy-line 55 wks laying hens were randomly allotted to 40 cages in 8 dietary treatments with 5 replicates (8 birds each). The experiment was conducted in a completely randomized design with 8 treatments involved a control group, four levels of hempseed (5, 10, 15, 20%), and three levels of hempseed oil (2, 4, 6%). The trial was done for 12 wks with three periods of 28 days. Eggs were daily collected and weighed. The highest egg production, egg mass and the lowest feed intake and the feed conversion ratio were observed in birds fed diets contained 6 % hempseed oil ($P < 0.05$). With increasing the levels of oil from 4 to 6%, the Haugh unit was increased at the third period ($P < 0.05$). The egg yolk color index was decreased when HS or HO added to dietary treatments. The hens fed hempseed oil had higher yolk index when compared to hens fed hempseed diets. Different treatments did not influence on plasma total protein. The highest levels of hempseed oil (6%) had significantly decrease blood plasma cholesterol, triglycerides, aspartate aminotransferase(AST) compare to the highest levels of hempseed (20% ; $P < 0.05$). The high density lipoprotein was higher in diets contained 6% hempseed oil as compared to the highest levels of hempseed (20%). Eventually, the lower level of hempseed (5%, 10%) had a suitable performance effect on laying hen. Hempseed oil had a better performance and egg quality compare to hempseed and it is suggested to be a useful oilseed in laying hen. It may be a suitable protein source to replace with soybean in laying hens.

Keywords: hempseed oil, performance, egg quality, blood parameter

S1- 0531 Effects of pomegranate pulp with enzyme on performance, carcass, abdominal fat and immune system of broilers reared under high temperature

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The present experiment was conducted to determine effects of pomegranate pulp on performance, carcass and abdominal fat of broilers reared under high temperature. 160 day-old broilers were divided to 4 dietary treatments included 0, 4, 7 and 10 percentage of pomegranate pulp (PP) with 0.05 % of Rovabio enzyme. The light schedule, humidity and temperature were used based on Ross catalog. The heat stress schedule included 6 hours 37 oC and normal temperature 21 oC were done from 28 day until 42 day. The results were revealed that broilers fed pomegranate pulp had higher body weight and lower FCR. The feed intake did not affected by dietary treatments. The relative weight of carcass, thigh, breast, intestine, liver, pancrease, and heart did not altered in response to dietary treatments. The diets contained 7 and 10% PP increased the relative weight of bursa fabricus, spleen. The antibody response against sheep red blood cells (SRBC) were higher in broilers received PP compared to control. The relative weight of abdominal fat was decreased in birds fed PP as compared to control. It is concluded that supplementation of PP to broiler diets may enhanced the broiler performance, increased the lymphoid organ such as Bursa and spleen and response of immune system. The PP diets may decrease the relative weight abdominal fat in broilers reared under heat stress conditions.

Keywords: pomegranate pulp, body weight, SRBC, lymphoid organ, heat stress

S1-0532 Plasma lipid profile, antioxidant status of broilers reared under high temperature fed pomegranate pulp and enzyme

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The present experiment was conducted to determine effects of pomegranate pulp on plasma lipid profile, antioxidant status of broilers reared under high temperature. 160 day-old broilers were divided to 4 dietary treatments included 0, 4, 7 and 10 percentage of pomegranate pulp (PP) with 0.05 % of Rovabio enzyme. The light schedule, humidity and temperature were used based on Ross catalog. The heat stress schedule included 6 hours 37 oC and normal temperature 21 oC were done from 28 day until 42 day. The results were revealed that broilers fed pomegranate pulp had lower plasma cholesterol, and LDL concentration as compared to control. The plasma triglycerides concentration did not affected by dietary treatments. The PP diets increased the HDL concentration. The total antioxidant capacity and TBARS indices were higher in 10 % PP and control respectively. The birds fed 7 and 10 % PP had lower plasma malodialdehyde concentration in plasma. It is concluded that supplementation of PP to broiler diets may decreased Cholesterol, LDL and increased HDL. The antioxidant status may improve in birds fed PP under heat stress.

Keywords: pomegranate pulp, TBARS, cholesterol, HDL, heat stress

S1-0533 Effect of replacing soybean meal with hempseed meal in post molting diets of laying hens

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The aim of this study was to recognize the effects of dietary hempseed meal (HM) on performance, egg quality and blood parameters of laying hens. A total of 320 Hy-line 55 wks laying hens were randomly allotted to 25 cages in 5 dietary treatments with 5 replicates (8 birds each). The experiment was conducted in a completely randomized design with 5 treatments involved: soybean meal 100% (SM) or zero percentage HM, 25% HM; 50% HM; 75% HM; and 100% HM replacement to SM. The experiment was done for 12 wks with three periods of 28 days. Eggs were daily collected and weighed. Egg production, Egg mean weight and feed intake in the birds fed to hempseed meal 100% were increased. This different compare with control group was significant ($P < 0.05$). Also, hempseed inclusion 100% led to significantly increase egg mass in last period of experiment compare with control group ($P < 0.05$). Albumin height (Haugh unit), yolk color index, yolk weight percentage and shell weight percentage were ameliorated when hens fed 100% HM compare with 100% SM. Replacing of HM was significantly decreased cholesterol, triglycerides and lipoprotein with low density compare with 100 % SM ($P < 0.05$). It is concluded that replacing of HM to SM may improve the performance of post molting layer and decrease the serum LDL and cholesterol of laying hens.

Keywords: aspartate aminotransferase, cholesterol, egg production, egg mean weight, haugh unit

S1-0534 Antioxidant status, plasma lipid of broilers fed diets contained pomegranate pulp with enzyme

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The objective of this experiment was conducted to determine effects of pomegranate pulp on plasma lipid profile, antioxidant status of broiler chickens. 160 day-old broilers were divided to 4 dietary treatments included 0, 4, 7 and 10 percentage of pomegranate pulp (PP) with 0.05 % of Rovabio enzyme. The light schedule, humidity and temperature were used based on Ross catalog. The results were revealed that broilers fed pomegranate pulp were decreased the plasma concentration of cholesterol, triglyceride, LDL. The diets contained 7 and 10 % PP increased the concentration of HDL. The total antioxidant capacity was gradually increased in broilers received different levels of PP. The birds fed PP had lower malondialdehyde or tiobarbituric acid reaction score (TBARS) as compared to control. It is concluded that supplementation of pomegranate pulp to broiler diets may decrease cholesterol, triglyceride, TBARS, and increase the HDL and total antioxidant capacity.

Keywords: pomegranate pulp, antioxidant, cholesterol, TBARS

S1-0535 Effect of different levels of dietary calcium sources (sea shells, calcium carbonate and limestone) on quality characteristics egg of commercial laying hens

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In this trial the effect of different levels of dietary calcium sources (sea shells, calcium carbonate and limestone) on Internal quality characteristics egg of commercial laying hens (shaver strains) were study. 280 hens at the age of 57 wks in a completely randomized design with 7 treatments, 4 replicates and 10 birds each were used in three periods of 28 days. The treatments were: 1. The samples with 100% shellfish powder, 2. samples with 100% calcium carbonate, 3. samples with 100% limestone, 4. samples with 50% powdered shellfish and 50 percent of limestone, 5. samples, with 50% limestone and 50% calcium carbonate, 6. samples with 50% shellfish and 50% calcium carbonate, 7. samples with 33% calcium carbonate, 33% shellfish and 33% limestone Statistical analysis shows that at the end of the second phase of tests experimental treatments were significantly affected. Statistical analysis of result revealed that the Haugh unit affected by the different treatments and treatment 1 and 7 have most significant difference compared to the other treatments ($p \leq 0.05$). Most yolk color was effected significantly by treatment 5 ($p \leq 0.05$). Different treatments were not showed significant effect on yolk and albumen percentage and yolk index. The result of this experiment indicate that shellfish or the use of shellfish with other resource has significant effect on internal quality of eggs during last phase of egg laying.

Keywords: calcium carbonate, egg quality, limestone, shellfish

S1- 0536 Effects of diets contained pomegranate pulp with enzyme on performance, carcass, abdominal fat and immune system of broiler chickens

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The present experiment was conducted to determine effects of pomegranate pulp on performance, carcass and abdominal fat of broilers reared under high temperature. 160 day-old broilers were divided to 4 dietary treatments included 0, 4, 7 and 10 percentage of pomegranate pulp (PP) with 0.05 % of Rovabio enzyme. The light schedule, humidity and temperature were used based on Ross catalog. The Body weight, feed intake and FCR were calculated at the end of three periods of starter, grower and finisher. The data were analyzed by SAS Software. The results were revealed that the feed intake and body weight did not affected by dietary treatments. The broilers fed pomegranate pulp had lower FCR as compared to control dietary treatment. The relative weight of carcass, thigh, breast, intestine, liver, pancrease, and heart did not altered in response to dietary treatments. The diets contained gradually levels of PP increased the relative weight of bursa fabricus, spleen. The antibody response again sheep red blood cells (SRBC) were gradually enhanced in broilers received PP compare to control. The relative weight of abdominal fat was decreased in birds fed PP as compared to control. It is concluded that supplementation of PP to broiler diets may enhanced the broiler FCR, increased the lymphoid organ such as Bursa and spleen and response of immune system. The PP diets may decrease the relative weight abdominal fat in broilers chickens.

Keywords: pomegranate pulp, FCR, SRBC, lymphoid organ

S1- 0539 Effect of levels of methionine supplementation in soaked-and-boiled *Mucuna sloanei* in Turkey poult's diet

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The objective of this study was to develop a high quality animal protein at reduced cost through the use of alternative feedstuff (*Mucuna sloanei* seed meal). One hundred and fifty day-old B- Not breed turkey poult's were used to assess the quantitative replacement of soybean meal with soaked- and-boiled *Mucuna sloanei* seed meal (SBMSSM) with or without methionine supplementation. Ten birds per treatment were replicated thrice in a completely randomized design .Diet 1 was control (0% SBMSSM), diet 2 had 5% SBMSSM quantitatively replacing soybean but without methionine supplementation, Diets 3 - 5 had 5% SBMSSM quantitatively replacing soybean but with 0.1, 0.2 and 0.3% methionine supplementation respectively. Feed and water were given ad-libitum for 56 days. The crude protein and gross energy of the test feedstuff were 23.74% and 3.40kcal/kg respectively. The anti-nutritional factors were L - Dopa (0.76%), tannin (0.13%) and hydrogen cyanide (1.37mg/kg). Growth performance values showed significant differences ($P<0.05$) among the treatment means. The feed conversion ratio of birds placed on diet 5 was the least. Haematological studies showed significant differences ($P<0.05$) for haemoglobin, packed cell volume and mean corpuscular haemoglobin concentration but they all fell within the normal range established for turkey poult's. There were significant differences ($P<0.05$) for urea, albumin and total protein values. Urea value obtained for birds placed on diet 5 was significantly lower than all other treatment means. Birds placed on diet 5 and diet 1 were statistically similar for the values of albumin and the total protein. Birds placed on diet 5 had the least cost/kg weight gain and highest gross margin; making it more economically viable among others. Diet 5 (0.3% methionine supplementation) enhanced a high quality animal protein production at reduced cost. This will lead to an increase in animal protein intake globally. It is therefore recommended.

Keywords: methionine, *mucuna sloanei*, soybean, turkey

S1-0540 The effect of *Fusarium* mycotoxins on cytochrome P450 enzymes and drug transporters at expression and functional level in broiler chickens

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Cytochrome P450 (CYP450) drug biotransformation enzymes and multidrug resistance (MDR) proteins, as P-glycoprotein (P-gP), may influence drug disposition processes. The aim of the present study was to evaluate the effect of chronic exposure to the mycotoxins deoxynivalenol (DON) and/or fumonisins (FBs), at contamination levels approaching EU guidance levels for feed, on intestinal and hepatic CYP450 enzymes and MDR proteins in broiler chickens. One-day old broiler chickens (n=64, Ross 308) were fed one of the four experimental diets for 15 days: control feed, DON contaminated feed, FBs contaminated feed, or DON + FBs contaminated feed. mRNA expression of CYP450 enzymes (CYP3A37, CYP1A4 and CYP1A5) and drug transporters (MDR1 and MRP2) were determined using qRT-PCR. A significant up-regulation of CYP1A4, CYP1A5 and MDR1 was observed in the jejunum of chickens fed a FBs contaminated diet. MDR1 was significantly upregulated in the ileum of chickens receiving DON+FBs contaminated feed. Consequently, the use of drugs that are a P-gP substrate, such as enrofloxacin, in the presence of FBs could result in a reduced oral bioavailability and systemic concentration. Therefore, the aim of the second part of this study was to investigate the impact of a FBs contaminated diet on the oral absorption of enrofloxacin. Following feeding during 2 weeks either a control diet or a FBs contaminated diet, 8 chickens per group were administered a bolus of enrofloxacin (10 mg/kg BW) directly in the crop. Subsequently, blood samples from the leg vein were taken at different time points after administration. A small but significant decreased area under the plasma concentration time curve and an increased clearance of enrofloxacin was observed for enrofloxacin and its major metabolite ciprofloxacin in chickens fed a FBs contaminated diet. These findings suggest that concurrent administration of drugs with FBs contaminated feed might alter the pharmacokinetics of drugs such as enrofloxacin.

Keywords: cytochrome P450, deoxynivalenol, fumonisins, P-glycoprotein, enrofloxacin

S1-0542 Influence of nanosilver zeolite on growth response, intestinal morphology and blood parameters of broiler chickens

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The experiment was carried out to investigate the effect of nanosilver zeolite, zeolite and flavomycin on growth performance, jejunal mucosal morphology and some blood parameters of broiler chickens. A total of 288 day-old un-sexed Ross 308 broiler chicks were allocated into 4 treatments, with 4 replicates and 18 birds per replicate by employing a completely randomized design. Dietary treatments were control with no additives, nanosilver zeolite (NSZ), zeolite (Z) and flavomycin (F). During the experimental period (0-42 days), adding NSZ and Z to the diet significantly decreased weight gain of the birds ($P < 0.05$), while had no effect on their feed intake, feed conversion ratio and mortality. There were no significant differences between performance parameters of chickens receiving control and F treatments. None of jejunal mucosal morphology parameters including villus height, crypt depth, villus surface area, villus height to crypt depth and muscle layer thickness was not significantly affected by dietary treatments. Supplementation of diets with NSZ and F significantly decreased sera total and LDL-cholesterol concentrations ($P < 0.05$). Triglyceride and VLDL-cholesterol levels were reduced by all dietary treatments ($P < 0.05$). The treatments had no significant effect on serum HDL-cholesterol concentration. However, NSZ and Z decreased the concentration of serum glucose ($P < 0.05$). It can be concluded that NSZ had adverse effect on broiler growth response and no positive effect on intestinal health. The reduction of some blood biochemical parameters might be related to liver damage induced by nanosilver fraction of the additive. Hence, further studies need to be done in this area.

Keywords: nanosilver zeolite, zeolite, flavomycin, intestinal morphology, blood parameters, broiler chicken

S1-0543 Chemical composition and true metabolizable energy content of corn co-products in cecectomized and intact adult roosters

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The objective of current study was to determine chemical composition and true metabolizable energy corrected to zero nitrogen balance (TMEn) content of some corn co-products in cecectomized and intact adult roosters. Six corn co-products (CGM, CGG, CB, CMR, CS and AG) were obtained from the producer company in Iran. DM, CP, EE, Ash and CF were determined by the standard methods and GE with Parr adiabatic calorimeter bomb. TMEn values of corn co-products were determined by Sibbald's method with using forty intact and sixteen cecectomized adult white leghorn roosters. Adult White Leghorn roosters that were approximately 50 wk of age were used for this research. They were adapted for a 10 d preexperimental period and were fed with a basal diet with 3200 Kcal ME/Kg and 12 % CP. The energy content was determined with an adiabatic calorimeter (Parr Instrument Company, Moline, IL). Nitrogen content of feed and excreta samples was determined with a nitrogen analyzer (2300 Kjeltex Analysis Foss). The effect of bird type on TMEn values was subjected to statistical analysis with using Student's ttest. CP content of corn gluten meal, corn germ meal, corn run mill, corn bran, corn starch and amino gluten meal were 57.48, 20.16, 3.70, 11.60, 0.53 and 39.98 percent, respectively. EE values varied from 2.86 percent in amino gluten meal to 23.63 percent in corn germ meal. GE values of corn gluten meal, corn germ meal, corn run mill, corn bran, corn starch and amino gluten meal were 5170, 4933, 4159, 4466, 4158 and 4004 Kcal/kg. TMEn values of corn gluten meal, corn germ meal, corn mill run, corn bran, corn starch, corn germ meal and amino gluten meal were 3507, 2174, 3248, 897, 2934 and 1909 Kcal/kg, respectively. TMEn values of corn germ meal, corn gluten meal and corn bran were 2093, 3466 and 667, kcal/g in cecectomized roosters, respectively. It was concluded that some corn co-products which produced in Iran, may have good potential in poultry feeding.

Keywords: corn co-product, adult cecectomized rooster, TMEn, nitrogen

S1-0544 The Effect of Mentha Pulegium, probiotics and antibiotics on fat and protein digestability in broiler chickens

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In this study the effects of Mentha Pulegium, probiotics and antibiotics on fat and protein digestability were studied. 600 Ross 308 broiler chicks in a completely randomized design with 10 treatments, 5 replications and 12 birds in each experimental unit were reared for 42 days. Treatments consisted of: 1) control, 2) level of 0.015 % antibiotics, 3) level of 0.03% antibiotics, 4) the level of 0.05% antibiotics, 5) level of 0.1% probiotic, 6) level of 0.2% probiotic, 7) 0.3% probiotic, 8) of 0.03% Mentha Pulegium, 9) of 0.05% Mentha Pulegium, 10) of 0.07 percent Mentha Pulegium. Statistical analysis of the results showed that The highest percentage of digestible protein appeared when probiotic 0.3% added to ration ($P < 0.05$). The highest percentage of digestible lipid belong to Mantha Pulegium 0.03% which was a significant different compare to other treatments. Overall the result indicated Mentha Pulegium and Probiotic led to improve a digestible material nutrition eventually both of them provide better performance compare with antibiotic.

Keywords: antibiotics, mentha pulegium, probiotics, broilers, digestability

S1-0545 Fatty acid profile of broiler meat affected by dietary fat sources

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This study was performed to determine the relationship between fatty acid (FA) profiles of the diet and that of broiler meat. Fat sources were soybean oil (SBO), hydrogenated soybean oil (HSBO), acidulated soapstocks (ASO), and recycled soy oil (RSO) in comparison with a control diet without additional fat. Five dietary treatments, using 4 fat sources and a control diet, were prepared. A total of 400 Ross 308, from 1 to 42 days of age, were placed in 20 pens (20 birds/pen) and 4 pens were randomly assigned to one of the 5 diets. Meat samples were collected after 42 d on trial. The results showed that the fat sources had substantial differences in FA profile so that Oleic acid was the most monounsaturated FA in HSBO (52.5%), while the ASO and RSO had similar content (34% and 33%, respectively) and lowest in SBO (24%). Linoleic acid was the most FA in SBO (50%) and ASO (40%). Its values were 11% and 32% in RSO and HSBO, respectively. The content of linolenic acid in SBO was about twice that of ASO (6 vs. 3%), but its amount was negligible in other fat sources. The results showed that the FA profile of fat sources was depending on the origin source and type of operations performed on it. Fatty acid profile of meat was influenced by that of diet ($P < 0.05$). Hydrogenated SBO caused the higher C16:0 and lower C18:2 contents compared to birds fed SBO diet ($P < 0.05$). C18:3 was higher ($P < 0.01$) in the hens fed SO than those fed RSO, ASO or HSBO. The results showed that meat FA profile was affected by the FA profile of the fat source used in diet.

Keywords: broiler, linoleic acid, meat, fatty acid profile, soybean oil

S1- 0548 The effects of a microbial phytase and polyethylene glycol in sorghum based diet on growth performance and meat oxidative stability of broiler chickens

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The present study was conducted to investigate the effect of a dietary microbial phytase and polyethylene glycol (PEG) supplementation in sorghum based diet on growth performance and meat oxidative stability of broiler chickens using 400 one-day old Ross 308 chicks by employing a completely randomized design with 5 treatments and 4 replicates. Dietary treatments including a corn based diet with no additive (control), sorghum based diets with no additives or with phytase, PEG or phytase + PEG were fed to birds for a period of 42 days. Through the experimental period, a similar growth performance was observed for birds on the control and sorghum based diet with no additive. The additives had no effect on feed intake and feed conversion ratio while increased broiler weight gain ($P < 0.01$). Increased gizzard digesta pH value was also observed by phytase or PEG adding to sorghum based diet ($P < 0.05$). Supplementation of phytase and PEG, alone or combined, increased ash content of tibia bone ($P < 0.01$). The amount of malondialdehyde in breast and thigh meats of broilers fed with sorghum with no additive and supplemented with phytase decreased, but it was increased by the supplemental PEG ($P < 0.001$). Phytase declined the cost of feed per kg of live weight and increased gross profit per bird ($P < 0.05$). Based on the findings, sorghum can be replaced for corn of broiler diets and its nutritive value can be improved by phytase or PEG supplementation. However, to achieve high meat oxidative stability and economic broiler production using phytase is preferred.

Keywords: broiler chickens, polyethylene glycol, meat oxidative stability, sorghum, phytase

S1- 0550 Growth performance and apparent nutrient digestibility of broiler chicken fed shea butter cake meal with or without probiotic supplementation

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A forty nine day study was conducted to determine the effect of feeding graded levels of Shea butter cake meal (SBCM) with or without probiotic supplementation on growth performance and nutrient digestibility of broiler chickens. A total of 420 arbor acre day old birds were allotted to seven dietary treatments with six replicates of ten birds each in a completely randomised design. Diet 1 served as control containing 0% SBCM without probiotic supplementation while diet 2 to 7 contained 5, 10 and 15% SBCM with or without probiotic supplementation as replacement for maize. The level of probiotic supplementation was 0.5g/kg. The broiler chicks were housed in deep litter pens. The experimental diets and clean water were provided ad libitum. Records of feed consumptions and weight gained weekly were kept on a pen basis. Five broiler birds of similar weights were selected from each replicate for apparent nutrient digestibility housed in a metabolism cage. The proximate composition of the cake revealed it contains 93.3% dry matter, 13.3% crude protein, 21.0% ether extract and 10.00% crude fiber. The results showed significant ($P < 0.05$) treatment effect on feed intake, weight gain and feed conversion ratio. The performance of birds were significantly ($P < 0.05$) improved by probiotic addition. The feed cost/kg and cost/kg gain was lower on shea butter cake based diets than the control group. Crude protein, crude fiber, ether extract and nitrogen free extract digestibility differed significantly ($P < 0.05$) among the different treatments. Apparent nutrient digestibility was observed to be higher in broiler birds fed probiotic supplemented SBCM than those fed SBCM without probiotic addition. It can be concluded that Shea butter cake is a potential feed ingredient as an alternative to expensive maize and probiotic supplementation enhanced its utilization with improved performance and digestibility of nutrients in broiler birds without any adverse effect.

Keywords: performance, nutrient digestibility, shea butter cake, probiotic, broilers

S1-0551 Evaluation of elusieve processing on the TMEn content of common feed ingredients used in commercial poultry diet formulation

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Due to costs associated with feed and feed manufacture, it is important to explore technology that may enhance the nutritional value of feed ingredients to optimize broiler performance. One technology of interest is the Elusieve process, which has the potential to remove fiber from a wide variety of feed ingredients using a combination of sieving and air classification. A companion study demonstrated the Elusieve process to increase the true amino acid digestibility of many amino acids for the enhanced fractionations of corn and 10% Crude Fat (CF) corn distiller's grains and solubles (DDGS); however, results were not consistent. The objective of the current study was to identify any changes in TMEn for ingredients that have been subjected to the Elusieve process; beginning corn, soybean meal (SBM), 10% CF DDGS, and 15% CF DDGS TMEn were to be compared to the resulting fractionations of ingredients (proposed enhanced and proposed fiber). White leghorn roosters were fasted for 24h and individually precision fed a 30g sample of each ingredient with 4 replications per fractionation (including endogenous controls) in a randomized complete block design. Excreta was collected at 48h post feeding and analyzed to determine TMEn. Beginning (un-fractionated) and the proposed enhanced fractionations of corn ($P<0.001$) and SBM ($P=0.023$) demonstrated an improved TMEn (approximately 1,500 and 400 kcal/kg, respectively) compared to fiber fractionations. However, proposed enhanced fractionations were not improved to values greater than beginning ingredients. Also, 10% CF DDGS demonstrated the highest TMEn values for the beginning ingredients with proposed enhanced fractionations performing intermediate ($P=0.001$). Elusieve did not demonstrate an effect on the TMEn content of 15% CF DDGS. These data suggest that the Elusieve process may not be feasible in a commercial setting unless a premium price could be obtained for the proposed fiber fractionation.

Keywords: TMEn, Elusieve, nutrient availability, feed manufacture

S1-0552 Composition of organic and conventionally grown poultry feed ingredients

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We hypothesized that organic and conventionally grown feed ingredients may have different composition and may have an effect on diet composition. We compared the composition of corn and soybean meal with corn used in conventional poultry feeds. The crude protein (CP) for the conventional corn was 8.15%, whereas methionine and cysteine were 0.17 and 0.19% respectively. The total sulfur amino acid (methionine + cysteine) for the conventional corn was 0.35% whereas that of organic corn was 0.28%. Whereas, lysine content was similar, threonine, tryptophan, isoleucine, arginine and valine content of the organic corn was lower than that of the conventional corn. The conventional soybean meal had a crude protein content of 47.67% compared to 47% of the organic soybean meal. The methionine and cysteine contents were 0.65 and 0.74% respectively, which were higher than the organic soybean. Similarly, lysine content of the conventional corn was 3.0% which was also higher than that of the organic ingredient. The threonine, arginine, isoleucine and valine content of the conventional soybean were 1.86, 3.52, 2.16 and 2.26 % which were all higher than the 1.74, 3.33, 1.78 and 1.93% from the organic soybean. The organic soybean had 0.70% tryptophan compared to 0.65% from the conventional soybean. The industrially produced flaxseed has 0.35 and 0.42 methionine and cysteine, respectively compared to the 0.38 and 0.34, respectively in the organic samples. Similar variability was found in other analyzed feed ingredients. Ingredient composition values from feed ingredient grown under conventional management practices may not be suitable for synthesizing organic poultry feeds.

Keywords: organic, amino acids, feed ingredients

S1-0553 Response of growth performance and intestinal microflora to sugar cane bagasse and corn particle size

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This study was conducted to evaluate growth performance and ileal microflora in response to sugar cane bagasse (lignocellulose) and corn particle size. Three hundred and thirty six Ross 308 male broilers were assigned in a 2×2 factorial arrangement of treatments with 2 particle sizes (coarse and fine) and 2 levels of a lignocellulose (0%, 2%). Each treatment had 6 replicate pens of 14 birds from 0-24 d. Feed conversion ratio (FCR), weight gain and feed intake were measured from 0-10 and 10-24d. Six groups of bacteria, i.e., *Bifidobacterium* spp., *Lactobacillus* spp., *Clostridium* spp., *Salmonella* spp., *Enterobacteriaceae*, and total anaerobic bacteria, were quantified from ileal digesta on d 24 using the 16S ribosomal primer assay. On d10, weight gain and feed intake were significantly higher in birds fed finely ground corn than those fed coarsely ground corn. FCR was impaired in birds fed diet containing 2% of a lignocellulose compared to the birds without bagasse treatment during d 0-10. No interactions were observed between particle size and sugar cane bagasse in early age. During d 10-24, however, significant particle size \times bagasse interactions were observed for FCR. The birds fed coarse corn with 2% bagasse had better FCR than those fed coarse corn without bagasse. However, this is not the case when fine corn was fed. Similarly, coarse corn improved FCR compared to fine corn only when 2% bagasse was applied. During d 10-24, birds fed diet with bagasse were heavier than those fed diet without it. Birds fed coarsely ground corn diet had a lower number of *Clostridium* than those fed finely ground corn. Bagasse had no effect on microflora. These findings suggest that while finely ground corn without supplemented fibre source is beneficial to broilers in the early age, feeding birds with coarsely ground corn supplemented with lignocellulose structural fibres improves the performance during the grower phase.

Keywords: lignocellulose, sugar cane bagasse, performance, microflora

S1-0555 The effect of citrus pulp on the lipid profile, some blood parameters and immune system of broiler chickens under heat stress conditions

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This study was conducted to evaluate the effect of feeding different levels of Citrus pulp on lipid profile, some blood parameters and immune system of broiler chickens. 160 commercial broilers (Ross 308) were randomly divided to four treatments (with four replicates and 10 birds each). The treatments were 4 levels including 0, 2.5, 5 and 7.5% of Citrus pulp. The heat stress induced in the last two weeks of experiment by increasing the temperature to 37 ± 1 °C for 6 hours per day. The results showed that the addition of Citrus pulp in broilers under heat stress did not have significant effect on concentration of cholesterol, LDL and triglycerides. Citrus pulp on the blood enzymes activities such as AST and ALT and antibody titer against SRBC had no significant effect. Therefore, the Citrus pulp may be added to diets without adverse effects on lipid profile, blood enzymes activities and immune system of broilers reared under heat stress condition.

Keywords: citrus pulp, heat stress, broiler, immune system, enzyme activity

S1-0556 Effect of different levels of Berberis Vulgaris pulp in broiler chicken diets on performance and carcass traits under heat stress conditions

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This study was conducted to evaluate the effect of feeding different levels of Berberis Vulgaris pulp on performance and carcass traits of broiler chickens. 160 commercial broilers (Ross 308) were randomly divided to four treatments (with four replicates and 10 birds in each replicate). The treatments were 4 levels including 0, 2.5, 5 and 7.5 of Berberis Vulgaris pulp. The heat stress induced in the last two weeks of experiment by increasing the temperature to 37 ± 1 °C for 6 hours per day. The results showed that the addition of Berberis Vulgaris pulp in broilers under heat stress did not have significant effect on body weight and feed intake and feed conversion ratio. Berberis Vulgaris pulp on the weight of the liver, thymus and abdominal fat had no significant effect. Therefore, Berberis Vulgaris pulp may not have adverse effects on performance and relative weight of carcass components and It could be supplementation to broiler diets reared under heat stress condition.

Keywords: broilers, berberis pulp, performance, heat stress

S1-0557 Optimal economic intake of isoleucine, valine and tryptophan for broiler breeder hens

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The objective of this study was to calculate the optimal economic intake of valine (Val), isoleucine (Ile) and tryptophan (Trp) for broiler breeder hens using the Reading Model. Sixty four hens in each amino acid (AA) assay were used. A completely random design was used, which consisted of eight treatments, eight replicates, and one hen per cage. The diets were formulated by dilution technique using one summit diet and one nitrogen (N)-free diet, resulting in AA levels that ranged from 1.97 to 9.85, 1.97 to 9.85, and 5 to 2.5 g/kg of Val, Ile, and Trp, respectively. Each experiment lasted nine weeks (five weeks of adaptation and four weeks for data collection). The amino acid intake, egg output (EO) and body weight (BW) were adjusted using a Reading model. The models generated to predict Val, Ile and Trp intake were: $\text{Val} = 12.5 \times \text{EO} + 41 \times \text{BW}$, $\text{Ile} = 9.9 \times \text{EO} + 20 \times \text{BW}$ and $\text{Trp} = 3.1 \times \text{EO} + 5 \times \text{BW}$, where EO=egg output (g/bird day) and BW=body weight (kg). Based on the models, birds with 3.5 kg of BW and 50 g/d of EO the flock require 768, 565 and 172 mg/bird per day of Val, Ile and Trp, respectively. The optimum economic intake was calculated at 837, 604 and 192 mg of Val, Ile and Trp/bird per day, respectively. The Reading Model can be used to estimate the optimum amino acid intakes for hens under different genetic and economic scenarios and depending on the ingredients available and their prices, the marginal cost of each amino acid will vary.

Keywords: amino acid, body weight, dilution technique, egg output, population response, Reading model

S1- 0558 Toxicopathological and Growth promoting effects of Sangrovit® in growing broilers

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Sanguinarine is a plant extract obtained from *Macleayacordataplant* belonging to family *Papaveraceae*. The present experimental study was planned to investigate the growth promoting of Sanguinarine available as commercial product with brand name of sangrovit®. One hundred day old broiler chicks were divided into 5 equal groups (A-E). The group E was kept as control group, while group A sangrovit @ 1 gm/10 lit drinking water (DW) 24 hours daily, group B sangrovit @ 1 gm/10 lit for 12 hours daily, group C 50 mg/kg feed, group D 1 gm/5 lit DW 24 hours daily. Parameters studied included clinical signs, feed intake, body weight gross lesions, hematology and serum chemistry. The visceral organs were fixed in 10% neutral buffered formalin for histopathological studies. The data thus obtained was subjected to analysis of variances (ANOVA) test. Birds administered with Sangrovit 1 gm/5 lit were depressed, less attractive towards feed, water and loose drooping were observed. Similar behavior was observed in the last two weeks in the group given Sangrovit 1 gm/10 lit. Mortality in group A was 25%, while in group D 35%. Feed intake of group D was significantly lower than the control group E. The body weight of group B and C were significantly higher than the control group. In serum biochemical parameters total protein and Globulin were significantly higher in groups C and D as compared to control group. Creatinine of all the groups were non significantly different from the control group. Urea of groups B, C and D were significantly higher than the group. Alanine aminotransferase (ALT) was lower in C group. From the above mentioned findings of the present study it is concluded that sangrovit should be used @ 1 gm/10 lit through drinking water 12 hours daily or 50 mg/kg feed. At this dose it is an excellent replacement of antibiotic growth promoters (AGPs)

Keywords: kidney, liver, pathology, intestine, serum, chemistry

S1-0560 Effects of whole sweet potato tuber flour inclusion in cockerel diets on performance, organs and haematological indices

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Feed resources like sweet potato are speculated to be unutilized, wasted, used sparingly or inefficiently used in livestock feed especially in Africa and Asia continents where it is produced in high quantity annually. Thus, this study was targeted at evaluating the effects of graded levels of whole sweet potato tuber flour (WSPTF) on cockerels' performance characteristics. Cockerels weighing 2 - 2.5kg at 10 months old were randomly allotted to four treatments with five replicates. The treatments were designated as Diet A (0.0% WSPTF), Diet B (10.0% WSPTF), Diet C (15.0% WSPTF) and Diet D (20.0% WSPTF). Although, 160g commercial grower mash (Supreme®) was earmarked for each cockerel, at point of feeding 0g, 16g, 24g and 32g were replaced with the WSPTF. Clean drinking water was offered to the cockerels that were housed together based on the treatment in deep litter pens which were cleaned regularly to maintain good hygiene. After 10 weeks, data on live weight, dressing percentage, feed and water intake, kidney, gizzard and liver weight as well as red blood cell, white blood cell and lymphocytes were obtained. It was shown that feed intake, liver and gizzard weight, red blood cell and lymphocyte values were statistically different ($P < 0.05$) among the treatments. While feed intake was highest ($98.1 \pm 13.3g$) in the control treatment (Diet A), live weight, water intake and dressing percentage values varied between $2,560 \pm 0.05$ and $2,640 \pm 0.06g$, 0.28 ± 0.08 and $0.30 \pm 0.06L$ as well as 86.4 ± 0.25 and $87.6 \pm 0.05\%$. Liver and kidney weight values were highest ($2.34 \pm 0.14g$ and $1.22 \pm 0.14g$) in Diet B but gizzard weight was highest ($27.6 \pm 0.46g$) in Diet D. Red blood cell and lymphocyte values were highest ($4.23 \pm 0.1 \times 10^{12}/L$ and $92.9 \pm 0.26 \times 10^2/dl$) in Diet B whereas, white blood cell was lowest ($1.16 \pm 3.08 \times 10^9/L$). Meanwhile, all these values were within the normal ranges reported in healthy cockerels of similar age and weight thus, WSPTF may be a veritable feed resource in poultry.

Keywords: haematology, organs, potato, poultry nutrition

S1- 0561 Combined use of dietary probiotic and acidifier for the production of antibiotic free broiler

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The effect of feeding probiotic (*Bacillus subtilis* and *Bacillus licheniformis*), acidifier (Salstop), antibiotic growth promoter (AGP) or probiotic plus acidifier was investigated in commercial broiler. A total of 400 (four hundred) Cobb-500 day-old straight run chicks were randomly distributed to 5 (five) different dietary groups having 4 (four) replications each. The number of birds in each replication was 20. The five dietary groups were as: control (basal diet); basal diet containing AGP at a level of 20g/100kg; basal diet containing probiotic at a level of 200g/100kg, acidifier at a level of 200g/100kg; and an equal amount of probiotic plus acidifier (200g/100kg). Broilers that received either probiotic, acidifier or a mixture of probiotic and acidifier (1:1) exhibited higher body weight gain, lower feed conversion ratio (FCR) and higher cost-effectiveness compared with the broilers fed on control diet ($P < 0.05$). However, feeding of diet containing both probiotic and acidifier resulted in the highest growth rate and net profit in all dietary regimen. Broilers fed on probiotic and acidifier in a mixture had FCR similar to other treatment groups ($P > 0.05$). It is concluded that the diet containing probiotic-acidifier mixture seems to be more cost-effective in promoting growth performance of broilers, as an alternative to the AGP, compared to the use of probiotic or acidifier alone in the diet.

Keywords: probiotic, acidifier, antibiotic growth promoter, broiler

S1- 0562 Performance of broiler chickens on wheat-based diets supplemented with novel carbohydrases

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The objective of this study was to assess the response of broiler chickens offered wheat-based diets and supplemented with varying levels of xylanase and glucanase (AB Vista, UK). A total of 600 male Ross 308 broiler chickens were randomly assigned to a 3 (none, low and recommended xylanase levels) \times 3 (none, low and recommended glucanase levels) factorial array in a completely randomized design. A nitrogen free diet (NFD) treatment was also included in order to assess endogenous nitrogen secretion (ENS) and standardized ileal amino acid digestibility (SIAAD). The nine main treatment combinations were replicated 6 times with 10 birds per replicate and each diet was fed ad libitum for 35 days in three phases - starter (1-10 days), grower (11-24 days) and finisher (25-35 days). The NFD was introduced to the relevant group between 19 and 24 days. Birds were raised in floor pens system. Data on feed intake and body weight were collected on 10, 25 and 35 days on pen basis while feed conversion ratio (FCR) was calculated and corrected for mortality. At 35 days, two birds per pen were randomly selected, slaughtered by cervical dislocation and processed for assessment of meat parts. The general linear model procedure was used to analyze the data (Minitab, version 17). Generally, the gross response exceeded the Aviagen specifications for Ross 308. Glucanase improved ($P < 0.05$) the FCR of broiler chickens offered wheat-based diets during 1-24 and 1-35 days of age. However, there was no change ($P > 0.05$) in feed intake and body weight in response to xylanase and glucanase. The relative visceral organ weight and meat parts yield were not affected by dietary treatment. Although there were no gross responses to the test enzymes, further analyses are underway, to determine their effects on ENS and SIAAD.

Keywords: glucanase, xylanase, feed conversion ratio, wheat, broiler

S1-0563 Effects of low maternal energy on carcass, meat quality and antioxidant capacity in Arbor Acres broilers

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To describe the effects of maternal dietary energy restriction on carcass, meat quality and antioxidant capacity in broilers, 400 Arbor Acres broiler breeder hens were fed diets with 4 ME levels (11.70, 9.36, 8.19, 5.85 MJ/kg) and All offspring hatched from 40 wk eggs were fed the same diets. Results showed that maternal energy level significantly affected egg weight and offspring body weight ($P<0.05$). Thigh muscle percentage of 28-d-old offspring, IMF content of 28-d-old chickens' chest muscle and 49-d-old chickens' thigh muscle were significantly affected by maternal energy level ($P<0.05$). Additionally, the drip loss of thigh muscle and meat color of 49-d-old offspring ($P<0.05$) were also affected. T-SOD, GSH-Px activity and MDA content in offspring muscles from 9.36 and 8.19 MJ ME/kg groups were significantly increased or decreased, respectively, 9.36 MJ ME/kg energy enhanced mRNA expression of GSH-Px in chest muscle compared ($P<0.05$). The results indicated that the effects of dietary energy restriction of broiler breeders on offspring production are significant. Compared with 11.70, 8.19, 5.85 MJ ME/kg group, 9.36 MJ ME/kg group had higher 49-d-old body weight and mRNA expression of GSH-Px in chest muscle, better antioxidant performance of chest and thigh muscle of offspring.

Keywords: low energy, carcass trait, meat quality, antioxidant capacity, broiler

S1-0564 Effects of dietary energy restriction during late laying period in broiler breeders on embryonic lipid deposition

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The objective of this study was to investigate the effects of a broiler breeder diet with low energy density on embryonic lipid deposition in yolk, embryo serum and tissues during the late laying period in Arbor Acres (AA) broiler breeders. Two hundred and seventy 20-week-old AA broiler breeders were randomly allocated into three treatment groups. One treatment group served as the control (ND). The other two treatments were trial groups whose diets were reduced in energy by 20% and 30% (LD20 and LD30, respectively). The experiment was conducted when the laying rate reached 5%. After artificial insemination at 60 weeks of age, 100 hatching eggs in each group were collected for 3 days and hatched. The results indicate that: compared with the ND group, the egg weight of the LD20 and LD30 group was decreased ($P<0.05$) before the hatching period, while the egg weight of the LD30 group was decreased ($P<0.05$) on day 13E. The egg content weight of the LD30 group on day 13E, 15E and 17E were lower ($P<0.05$) than that of the ND group. The embryo weight of the LD30 group on day 15E was lower ($P<0.05$) than that of the ND group. Yolk cholesterol at each embryonic day and CHO of thigh muscle on day 17E in the LD30 group were lower ($P<0.05$) than those of the ND group. Yolk gross energy and serum TG in the ND group were higher ($P<0.05$) than in other groups. Yolk crude fat on days 17E, 19E, and 21E and CHO in liver on days 15E, 17E, and 19E in the ND group were higher ($P<0.05$) than in other groups. Serum HDL-cholesterol and LDL-cholesterol in the LD30 group were higher ($P<0.05$) than in other groups. Serum cholesterol at each embryonic day and CHO in breast muscle on days 19E and 21E in the LD20 group were higher ($P<0.05$) than in other groups. These results indicate that maternal dietary energy restriction directly affected lipid metabolism in embryos during the later laying period.

Keywords: energy restriction, broilers, embryo, lipid deposition

S1– 0565 BTs improved the broiler performances showing potential as substitutes of antibiotics

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A total of 3200 day-old broilers were randomly separated into five groups. Each group included 640 chicks which were distributed into 16 slots and each slot included 40 chicks. The chickens in five groups were fed with five different diets, including unmedicated diets (negative control), 40ppm colistin + 20ppm BZ (positive control), 24ppm BTs, 48ppm BTs, and 25 ppm CAD, respectively. The experiment lasted to 42 days. During this period, the level of GH, IGF-I, T3 and T4 in serum were determined. The GH level of broiler in 24ppm BTs, 48ppm BTs and 25ppm CAD treatment group were significantly higher than those in the negative and positive control treatments on 11-day-old ($P < 0.001$). The IGF-1 level of broilers fed with diets containing 25ppm CAD was dramatically changed from 8 to 20 days. The T3 level of broiler from positive control and 24 ppm BTs treatment group were similar, whereas, T3 level of broiler from negative, 48ppm BTs and 25ppm CAD treatment group were significantly changes at 11 days. Subsequently, the concentrations of T3 in five treatment group trended to be uniform from 14 to 20 days. The concentrations of T4 were not significantly changed in five treatments at different ages. Average daily gain (ADG) and feed conversion rate (FCR) of broilers fed with BT cationic peptides diet were improved significantly during the starter period (0- 21 days). Collectively, our results suggested that BTs could improve the broiler performances by advancing the GH peak. BTs have potential to be utilized as growth promoters to alternate antibiotics.

Keywords: antibacterial peptide, cationic peptides, GH

S2–0002 Population studies of 14 registered Hungarian poultry breeds conserved in the 21st century

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The study aims to analyse the number of pedigree stocks (n), sex ratio (N_m/N_f), effective population size (N_e) and inbreeding rate (ΔF) within populations as well as the relationship between n , N_e and ΔF of 14 local Hungarian poultry breeds including Yellow Hungarian chicken (YHc), White Hungarian chicken (WHc), Speckled Hungarian chicken (SHc), Partridge Coloured Hungarian chicken (PHc), White Transylvanian Naked Neck chicken (WTc), Black Transylvanian Naked Neck chicken (BTc), Speckled Transylvanian Naked Neck chicken (STc), Hungarian Landrace Guinea Fowl (HLgf), Frizzled Hungarian Goose (FHg), Hungarian Goose (HUG), White Hungarian Duck (WHd), Wild Coloured Hungarian Duck (Wld), Copper Turkey (COt) and Bronze Turkey (Brt) from 2000 to 2015. The n of most breeds increased yearly. Average N_m/N_f of breeds studied can be ranged from 0.141 to 0.496. N_e varies widely from 92 to 2581 and generally higher in the period between 2011 and 2013 in comparison with the other periods of time. Average N_e/N of all breeds is higher than 0.427 and the highest was recorded in HLgf (0.851 in 2005- 2009). The lowest average ΔF of 0.031% and the highest of 0.458% was recorded in HLgf (2010-2015) and WHd (2005- 2009), respectively. Noticeably, the highest average $F\%$ of most breeds was obtained in 2005-2009. Only COt had gradual decline in the $\Delta F\%$ over studied periods. With the exception of HUG, the n correlates positively with N_e , and negatively with F ($P < 0.01$), which reflects the significance of n in conservation practice. The study suggests to monitor n , N_e and ΔF as frequently as possible and recommends a conservation strategy that minimizes ΔF by increasing N_m/N_f . The high n as well as sustainable subsidies are essential to eliminate any risk of dramatic decrease in N_e , thus assuring the safety of conservation programme of a breed. Based on effectiveness and reliability, the study would promote the use of Hungarian conservation programme as a model in practice.

Keywords: conservation, Hungarian poultry, inbreeding, population

S2-0003 Morphobiometrical diversity of the indigenous chicken's population in the Sudano-sahelian zone of Cameroon

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Studies were undertaken at rural, peri-urban and urban areas of the Sudano-sahelian agro-ecological zone of Cameroon to describe the variations in physical characters and some important feather and skin mutations observed in different populations of native chickens of the sudano-sahelian zone of Cameroon. According to accessibility, availability of chickens and willingness of farmers to give informations, thirteen villages were investigated from January to September 2010 in the Far-north and North regions using a structured questionnaire. 558 chickens were randomly selected and each of them was completely described by direct observation, weighing and body measurements according to FAO (1981) recommendations. The main results show that feather colour of local poultry of the Sudano-sahelian agro-ecological zone of Cameroon is variable, but dominated by the wild type and white having frequencies of 18.64% and 15.41% respectively, whereas the other colours of feather vary from medium to very small frequencies, as grey colour of feather (1.61%). Four genetic types were represented in this agro-ecological zone, namely: normally feathered (87.63%), Naked-neck (4.30%), crested (4.84%) and Frizzle chickens (3.23%). Heterogeneity of colours was also observed at the level of shanks; where white was the dominant colour (38.53%) and green (4.12%) the lowest frequency. The comb showed just two varieties: single (95.52%) and rose shape (4.12%). Sexual dimorphism was observed in all the traits with higher values recorded for males. The mean body weight of adult chicken, in the Sudano-sahelian agro-ecological zone of Cameroon is 1458 ± 329 g. Roosters are heavier (1588 ± 332) than hens (1323 ± 269 g). All the body measurement considered are significantly higher in roosters. The high variability of characters offers possibilities for selection of rustic and more productive breeds in the Sudano-sahelian agro-ecological zone of Cameroon

Keywords: local chickens, morphological, sudano-sahelian zone

S2-0006 Identification of SNPs in Myoz3 gene and their association with carcass traits in two chicken lines

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Myozenin 3 (Myoz3) belong to Myozenin family, which have a key role in Z-disc structural, muscle fiber differentiation and signal transduction. In present study, we aim to identify polymorphisms of Myoz3 and investigate their potential as valuable markers for MAS. Two broiler lines were selected for this study including Yellow Bantam (YB) and Avian. We randomly picked 70 individuals from each line. Multiple traits were measured. Total DNA was extracted from blood, four pairs of primers were designed to amplify all 5 exon of Myoz3. Genotypes were analyzed and the general linear model procedure of SAS (version 9.4) was used to analysis associations between the genotyped markers and carcass traits. We found 5 SNPs of Myoz3 in YB line and 3 SNPs in Avian line. Association analysis revealed that in YB line, SNP c.501C>T have a strong effect on FM (Femoral circumference), group with genotype CC was statistically advanced compare to those with CT genotype. While c.516C>T have a strong effect on both shank length and lightness (L*) value of breast muscle, group with CC genotype have a longer shank length and lower L* value of breast muscle. Meat quality is strongly depend on muscles fiber types, but Myoz3 is still poorly studied in chickens. To the best of our knowledge, this is the first analysis of polymorphisms of the MyoZ3 gene in chicken breeds. The current study demonstrates that the polymorphisms of Myoz3 in two chicken lines exist great difference. In present study, we find that polymorphisms of Myoz3 between two chicken lines is of great difference, and the heterozygous was only found in YB line but not in Avian line, suggesting that this gene may be has already under high selective pressure during phenotype based selection. Two valuable markers found in this study might be instructional for breeding and selection.

Keywords: Myoz3, SNPs, MAS, carcass traits

S2- 0007 Genetic variation of nine chicken breeds collected from different altitudes revealed by microsatellites

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Genetic polymorphisms of 20 microsatellites were investigated in nine local chicken breeds collected from low, middle and high altitudes areas in China (total number was 257) and their population genetic diversity and population structure were analyzed. All breeds were assigned into three groups, including the high (Tibetan chicken (T) and grey chicken (G), their altitude was above 1000 m); middle (Chengkou mountainous chicken (CK), Jiuyuan chicken (JY) and Peng county yellow chicken (PY), their altitude was between 500 and 1000 m), and low groups (Da ninghe chicken (DH), Tassel first chicken (TF), Gushi chicken (GS) and Wenchang chicken (WC), their altitude was below 500 m). We found 174 genotypes and 108 alleles via the 20 microsatellite primers, and the results showed that the number of alleles (Na) with a mean of 5.4; the average of polymorphism information content (PIC) was 0.571; the mean of expected heterozygosity (He) was 0.673; as for observed heterozygosity (Ho), the mean was 0.628. The AMOVA results indicated there was no significant genetic variation among different altitude groups (0% of the total variation) and showed high level of variation within individuals among populations (80.1%). The deficiency of heterozygosity relative to Hardy-Weinberg expectation (FIS) values with a mean of 0.001. The mean of gene flow (Nm) was 1.688. The mean of FST between paired populations was 0.166. Ninety percent of all loci deviated Hardy-Weinberg equilibrium. The genetic distance ranged from 0.232 to 0.782. Generally, the natural selection for the location with different altitude is not strong enough to significantly differentiate the populations in terms of microsatellite variation. Many local breeds in China don't match the high meat or egg yield requirements for commercial production, their population sizes were sharply decreased. Our results provided the genetic information for making conservation strategies for these populations.

Keywords: chicken; microsatellite; altitude; genetic variation; population structure

S2-0008 Comparison of growth patterns of four strains of commercial broiler chicken

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The objective of this study was to compare the growth pattern for body weight of some strains of commercial broiler chicken using a non-linear model, Gompertz function. Four hundred (400) one-day old chicks comprising of 100 each of broiler strains ST1, ST2, ST3 and ST4 were raised for a period of 49 days. The birds were fed with the same starter diet for the first 21 days and finisher diet for the last 28 days. At the end of each week, body weight measurement of the birds were recorded and fitted into a sigmoid curve to depict the growth pattern of the bird. Gompertz model was applied to describe the weight-age relationship in the birds and estimate: mature weight (A), rate of gain after hatch (B) and rate of maturity (C). The result showed that average body weight at hatch was highest in the ST3 strain (43.11) and least was observed in the ST2 strain (41.91), the highest A value (predicted mature weight) was observed in the ST2 strain (3568.2), followed by ST1 strain (3042.6) and the least value was obtained in ST3 strain (2923.8). Parameter B obtained in this study was highest for the ST1 strain (6.3473) and the least was obtained from ST3 strain (5.8270), a higher C (rate of maturity) value (0.3559) was predicted for the ST1 strain, this is followed by ST4, ST3 and the least value (0.3070) was the ST2 strain. The values of coefficients of determination (R²) were high for all the strains. However, there were corresponding lower mean square error values for all the strains. In conclusion, the ST2 strain was found to be late maturing and heavier at maturity while the ST1 strain was shown to have higher growth rate.

Keywords: growth curve, gompertz function, broiler strains

S2- 0009 Lentivirus- mediated RNA interference of MyoD gene in duck primary embryonic myoblasts

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The MyoD which belongs to the family of muscle regulatory factors (MRFs) play a fundamental role during myoblast proliferation, in the present study we assessed the function of MyoD by gene silencing in duck primary embryonic myoblasts. Three shRNAs, each targeting different region of the duck MyoD gene (GenBank accession no: NW_004676605) and a negative control shRNA were designed and synthesized. Duck primary embryonic myoblasts were prepared from 15-day-old Jingding duck embryos. The cells (1×10^6 cells/ml) were then seeded in 6-well plates and cultured in a 5% CO₂ incubator at 39°C. Lentivirus- mediated shRNA vectors targeting MyoD and shControl were transfected into duck primary embryonic myoblasts. The mRNA and protein expression levels of MyoD were detected using real-time PCR and western blotting, cell proliferation was assessed by MTT assays, and cell differentiation was assayed by photography. MyoD mRNA levels in shRNA1, shRNA2, and shRNA3 groups were reduced by 59.4%, 67.3%, and 66.9%, respectively, compared with control cells. Protein levels were reduced by 44.9%, 64.9%, and 50.6%; these results showed that three shRNA could significantly and stably reduce the expression of MyoD. Down-regulation of MyoD induced cell proliferation and inhibited differentiation, accompanied by a greater than two-fold down-regulation of MyoG, Myf6 and MSTN expression and up-regulation of Myf5 expression. MyoD affects the transcriptional activation of the various downstream target genes, which include the other MRFs genes (MyoG, Myf6 and Myf5) and MSTN gene (negatively regulate skeletal muscle growth). Myf5 appears to be functionally equivalent to MyoD in its capacity to convert cell to myogenic lineage, they are structurally related; in this study the mRNA level of Myf5 was up-regulated when successfully silencing MyoD gene in myoblasts cells. These results revealed the important role of MyoD in duck embryonic myoblasts.

Keywords: MyoD, shRNA, duck, myoblast, MSTN, MRFs genes

S2- 0010 Molecular cloning and expression analysis of the Clock gene in the hypothalamus of Zie goose during different stages of the egg-laying cycle

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Background: Clock gene deemed as the core gene of circadian rhythm. Its expression of protein CLOCK combined with BMAL1 into a hetero dimer. Gallus Clock gene plays an important role in the regulation of biological rhythms and embryonic development, which suggests that the Clock gene associated with reproductive traits in livestock and poultry. However, the mechanism of action of Clock gene regulating goose reproduction traits has not been reported. We cloned the part CDS sequence of the Clock gene and studied the expression of the gene in the hypothalamus of the Zie goose in the different stages of the egg-laying cycle. Method: The hypothalamus Clock mRNA level was tested by real-time fluorescence quantitative PCR method in each six geese at the stage of pre-laying period (January), early-laying period (early March), peak-laying period (early May) and late-laying period (late June). Result: The partial CDS (Length of 303bp) of Clock gene was obtained by Clone, which shared consistency with the Clock gene of gallus and anas in 99% and 96%, respectively. The results of qRT-PCR demonstrated that the expression of hypothalamus Clock mRNA increased from the pre-laying period to the late-laying period, reached its peak in the late-laying period, and the mRNA levels of Clock gene showed an exponential growth from January to June. The level of hypothalamus Clock mRNA in pre-laying period was the lowest. The level of Clock mRNA at early-laying period, peak-laying period and pre-laying period were significantly higher ($P < 0.01$) than the pre-laying period. Conclusion: The level of hypothalamus Clock mRNA is highly associated with the local sunshine durations (hours) in Zie goose. With the extending of sunshine duration, the level of hypothalamus Clock mRNA gradually increases. This research result will provide fundamental basis for elaborating the molecular mechanism of clock gene in regulating Zie geese reproductive trait.

Keywords: Zie goose; Clock gene; fluorescence quantitative PCR

S2-0012 The change of Nod1mRNA expression during Salmonella challenge in AA+ chicken

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Nod1 is a member of the NOD-like receptor family of intracellular proteins. Which mediate host recognition of bacterial peptidoglycan, and play a crucial role in the early stage of the innate immune response. The purpose of the present study was to analyse Nod1 expression profile in different tissues and different development stage, and to investigate the role of Nod1 signaling pathway in Salmonella infection in AA+ chicken. In this study, healthy AA+ chickens were purchased from National Chicken Genetic Resources (Jiangsu, China). Real-time PCR was used to quantify genes expression in chicken by the Stratagene Mx3000P QPCR system. The result showed that, in the one-day AA+ chicken, the Nod1 transcript was found to be expressed in a wide range of tissues. Which is highest expression in blood, moderate expression in heart and lung, low expression in spleen, fabricius, rectum, liver, thymus, small intestine, lung, brain, testical, ovary and kidney. The results showed that the chicken Nod1 expression pattern was from low to high and then to low in spleen of different development stage. At 28th day, the Nod1 expression level was higher than that of 1th, 14th and 42th development stage. After Salmonella pullorum and Salmonella enteritidis challenge, the mRNA expression level of chicken Nod1, Ripk2, NF- κ B, IL-1 and IL-8 were all increased, significant or highly significant difference with the control group at or after three days. The results indicated that the chicken Nod1 gene expression has the tissue-specific and development stage-dependence. And the results indicated that Nod1 signaling pathway may play an important role against salmonella infection in chicken, but the mechanism is not clear, more research need studied on it. We hope the data from this study may be useful for research on the function of the chicken Nod1 gene and breeding for disease resistance in birds.

Keywords: chicken, Nod1, salmonella, expression

S2-0013 Correlation analysis of tyrosinase (TYR) gene, agouti signaling protein (ASIP) gene and microphthalmia-associated transcription factor (MITF) gene on melanin deposition of White Silky Flow

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In order to clarify the molecular of melanin deposition in White Silky Flow and explore the relationship between mRNA expression of tyrosinase (TYR) gene, agouti signaling protein (ASIP) gene and microphthalmia-associated transcription factor (MITF) gene and melanin deposition in tissues of White Silky Flow. Sixteen (same male and female) 150 days White Silky Flows were picked out from the national conservation field in Putian city Fujian province, then extracted skin, muscle, liver, kidney, and gizzard. The quantitative real-time PCR was used to detect the expression level of TYR gene, ASIP gene and MITF gene in tissues of White Silky Flow, and the ultraviolet spectrophotometry was adopted to measure the melanin content in tissues for exploring the relationship between mRNA expression of the three genes and melanin deposition in tissues of Silky Flow. Significance test and correlation analysis were performed by SPSS13.0 software. Results showed that the mRNA expression of TYR, MITF and ASIP gene were detected in all tissues of Silky Flow, which were significantly different among tissues ($P < 0.01$). The mRNA expression of MITF and TYR gene in tissues presented a general trend: skin > kidney > gizzard > liver > muscle, which were consistent with the law of melanin deposition in Silky Flow. However, the mRNA expression of ASIP gene showed a contrary trend: muscle > liver > gizzard > kidney > skin. Correlation analysis indicated that the mRNA expression of TYR and MITF gene were positively correlated with the melanin content in tissues of Silky Flow ($P < 0.05$), while the mRNA expression of ASIP gene was negatively correlated with that ($P < 0.05$). It was speculated that high expression of TYR and MITF gene promoted melanin deposition while ASIP gene inhibited melanin deposition in Silky Flow. This research revealed the regulation mechanism of the 3 genes expression to melanin deposition in Silky Flow.

Keywords: White Silky Flow, melanin deposition, quantitative real-time PCR, correlation analysis

S2-0014 Hatching traits in four closebred flocks of Japanese quail at three ages selected for higher body weight in 4th generation using different selection strategies

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Present study evaluated effect of different selection strategies in four closebred flocks (CBF) at three ages on hatching results during 4th generation. Five hundred and forty Japanese quails already selected for higher body weight from four CBF (Major, Kaleem, Saadat and Zahid) at three ages (10, 12 and 14 weeks) were subjected to 3 selection strategies (i.e., pedigree, mass selection and random bred control). In pedigree selection 108 birds with higher body weight were selected with full pedigree record whereas in mass selection, 324 birds with higher body weight were selected to be the parents of next generation. However, in random bred control groups, 108 birds were selected without following any selection. The effect of selection strategies in parents of Japanese quails at 3 ages from 4 CBF were measured on its hatching results. Selection significantly influence hatchability, fertility, infertile egg, dead germ, dead in shell % and chick weight. It is concluded that selection has pronounced effect on hatching traits in Japanese quail.

Keywords: CBF, age, hatching traits, Japanese quail

S2-0015 Production performance in four closebred flocks of Japanese quail at three ages selected for higher body weight in 4th generation using different selection strategies

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Present study evaluated effect of different selection strategies in four closebred flocks at three ages on production performance during 4th generation. A total of 540 Japanese quails already selected for higher body weight from four CBF (Major, Kaleem, Saadat and Zahid) at three ages (10, 12 and 14 weeks) were subjected to 3 selection strategies (i.e., pedigree, mass selection and random bred control). In pedigree selection 108 birds with higher body weight were selected with full pedigree record whereas in mass selection, 324 birds with higher body weight were selected to be the parents of next generation. However, in random bred control groups, 108 birds were selected without following any selection. The effect of selection strategies in parents of Japanese quails at 3 ages from 4 CBF were measured on its production performance. Selection had pronounced effect on body weight, feed intake, egg weight, FCR, and Production %.

Keywords: selection, CBF, age, production performance, Japanese quail

S2-0017 A genome-wide association study identified major loci and region affecting body composition traits of chicken

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To identify SNPs that significant associated with body composition traits, a genome-wide association study was carried out using the 60K SNP beadchip in Jinghai yellow chicken. The experimental population was 212 female Jinghai yellow chickens. The blood was collected and the body weight (BW), body composition weight (CW), foot weight (FW), wings weight (WW), single breast muscle weight (BMW), single leg muscle weight (LMW), abdomen fat weight (AW), eviscerated weight (EW), semi-eviscerated weight (SEW) were weighed. DNAs were extracted and sent to DNA LandMarks Inc in Canada to genotype using 60 K SNP beadchip. Plink (v1.07) and GCTA (v1.24) were used to analysis the data. The general linear regression model (GLM) in PLINK were used in this study. The model was $Y = G\alpha + Pj\beta + e$. The results showed 25 SNPs reached 5% Bonferroni genome-wide significance. 15 out of 25 SNPs were located in a 1.9Mb (77.4-79.3Mb) region on GGA4, which was associated with BW, FW, WW, W, EW and SEW. Four SNPs associated with FW and WW were located on GGA4, 5 and 7, respectively. The remaining six SNPs were located on GGA1, 2, 12 and 15, and had effects on BMW, AW and AWP, respectively. Names of genes in the candidate regions with 1-Mb windows surrounding each significant SNP were obtained from Ensembl and NCBI. A total of 12 candidate genes were identified. SNPs located in FAM184B gene have strongest effects on BW, FW, WW, LMW, EW, and SEW. SNPs in GRIK1 gene were significant associated with BMW. SNPs in CACNA2D2 were significant associated with AW and AWP. The results above indicated that the region of 75.5-79.3Mb on GGA4 played an important role in regulating body composition traits of Jinghai Yellow Chicken. SNPs above and three genes of FAM184B, GRIK1 and CACNA2D2 might be important candidate markers and genes that had effect on body composition traits of Jinghai Yellow Chicken.

Keywords: chicken; GWAS; body composition; haplotype; FAM184B

S2- 0018 Genomic analysis reveals deletion of IHH as the cause of semi-lethal Creeper trait in the chicken

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The Creeper trait, a classical monogenic phenotype of chickens, is caused by a dominant semi-lethal mutation that expresses shortened extremities in development. The linkage of gene for Creeper and single-comb was the first reported autosomal linkage case in fowls and farm animals. However, little is known about the mechanism of semi-lethal Creeper trait formation. Here we have performed whole-genome sequencing and extensive analysis for targeting causative mutation underlying the Creeper trait. The deletion of Indian hedgehog (IHH) was only found in the Creepers and lethal embryos from whole-genome sequencing data. Large scale segregation analysis demonstrated that the deletion of IHH was fully linked with early embryonic death and the Creeper trait. IHH dominates cartilage differentiation, regulates chondrocytes proliferation and is necessary for bone formation. Expression analysis showed a dramatic expression difference between Creeper and wild-type chickens and much lower expression in Creepers as compared to wild-types. Our results strongly demonstrate that deletion of IHH is the causative mutation for the Creeper trait owing to IHH haploinsufficiency. Our findings emphasize the pivotal role of IHH in animal development and would provide a unique and valuable in vitro model for the study of IHH function and haploinsufficiency diseases.

Keywords: semi-lethal mutation, chicken, Creeper trait, IHH, haploinsufficiency

S2- 0019 Phenotypic characteristics and heredity of upright and pendulous comb in chicken

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Upright and pendulous combs commonly exist in most single comb chicken breeds. In the present study, the phenotypic characteristics and the heredity of upright and pendulous combs were analyzed. Phenotypic frequencies of upright (UC), partial pendulous comb (PPC) and complete pendulous comb (CPC) were investigated in five chicken breeds including Huainan chicken (n = 2208), Dongxiang chicken (n = 2829), Wenchang chicken (n = 1905), Jinghong layer (n = 1990), and Nongda-3 layer (n = 2863); the phenotypic frequencies of CPC and PPC ranged from 10.1% to 29.0% and 16.8% to 65.3 %, respectively. Four pure lines including Nongda-3 (n = 1893), Huainan (n = 1420), Wenchang (n = 1035) and Dongxiang (n = 1829) were used to measure the 300 d egg number of three comb phenotypes chickens. Results show that CPC hens produced more eggs than PPC hens in Nongda-3, Huainan and Wenchang breeds. Huainan breed (n = 600) were used to measure the growth of chickens with different comb phenotypes. CPC males were heavier than PPC males at 12 and 16 wk of age and CPC hens were heavier at 24 wk of age. Four types of mating were carried out (CPC ♂ × UC ♀, CPC ♂ × CPC ♀, UC ♂ × UC ♀, and UC ♂ × CPC ♀) to study the heredity of upright and pendulous comb (Huainan breed). Each type of mating involved 5 cockerels and 25 hens to construct 5 families. Chi-squared analysis showed that there was no significant difference in comb phenotypic frequency distribution between the offspring from UC(♂) × CPC(♀) and CPC(♂) × UC(♀), however, comb phenotypic frequency distribution differed between offspring from UC(♂) × UC(♀) and CPC(♂) × CPC(♀). These results suggesting that the upright or pendulous comb trait may influence chicken growth and egg production. Furthermore, the upright or pendulous comb may be influenced by several major effect loci or some minor effect loci in the autosome or both.

Keywords: chicken, upright and pendulous comb, growth, egg production, heredity

S2- 0021 Association between BM-PR-IB gene polymorphism and reproduction traits and its tissues expression characteristics in chicken

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To analyze the expression characteristics of BM-PR-IB gene in different tissues and the association between BM-PR-IB polymorphisms and reproduction traits in Jining Bairi chicken. Four hundred Jining Bairi chickens were used for the detection of SNPs in BM-PR-IB and the analysis of the relationship between the SNPs and the reproduction traits. Five Jining Bairi chickens were randomly selected for the tissue sampling of the liver (L), oviduct (S) and ovary (O) at the age of 19W, 23W and 40W, respectively. Also, all levels of follicles were obtained at the age of 40W. In BM-PR-IB gene, three SNPs were detected. They were C217039T (BR217039) in intron 2, T230065C (BR230065) in intron 6 and C233062T (BR233062) in intron 8. In BR217039 site, the Egg production at 43 W (E43) of E1E1 chickens was higher than E2E2 ones (P<0.05). In BR230065 site, the age at first egg (AFE) of F1F2 chickens was earlier than F1F1 ones (P<0.05). In BR233062 site, the significant differences of living weight at first egg (LWFE) (P<0.05), living weight at 43 W (LW43) (P<0.01) and EW43 (P<0.01) appeared between G1G1 chickens and G2G2 ones. The tissue expression for BM-PR-IB gene showed that the expression level in ovarian follicle was 4 mm> 6-8 mm> 15-19 mm> 23-29 mm> 33-34 mm in diameter. The mRNA level in follicles of 4 mm in diameter was significantly higher than that in follicles of 15-19 mm, 23-29 mm and 33-34 mm in diameter (P<0.01). In different developing periods, the expression level was 23 W> 19 W>40 W in liver and hypothalamus, and the mRNA level in 23 W was significantly higher than that in 40 W (P<0.01). In ovary, the expression pattern was 19 W> 23 W>40 W, and the mRNA level in 19 W was higher than that in 40 W (P<0.05). BM-PR-IB plays a vital role in the development of ovary and follicles, especially in the development of primary follicles.

Keywords: Gallus gallus, BM-PR-IB gene, reproduction traits, expression profiles

S2-0023 Improvement of indigenous chicken in Bangladesh

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Present research is a part of the long-term selection program being undertaken (i) to assess the performances of three indigenous chicken genotypes under intensive management (ii) to predict responses while improving 3 Indigenous Chicken genotypes. A total of 5945-day-old chicks comprising of 3 types of chicken namely Naked Neck (NN), Hilly (H) and Non-descript Deshi (ND) were hatched for foundation generation (G0), first generation (G1), second generation (G2) and third generation (G3) for this study. In every generation, selection was practiced at 40-week of age on the basis of an index comprising the parameter of age at first egg (AFE), body weight (BW), egg production (EP) and egg weight (EW). The data were analyzed by factorial arrangement in a CRD by General Linear Model (GLM) Univariate Procedure in SPSS computer program. Expected genetic progress due to selection in a generation for EP, EW, BW and AFE were estimated for foundation generation (G0), first generation (G1) and second generation (G2). Significant body weight differences among the genotypes were observed at all stages, with the highest body weight observed for Hilly (H) genotype. Among the three Indigenous Chickens, ND had significantly ($p < 0.001$) better value in average number of eggs production HDEP% (51.4 ± 0.4) and HHEP% ($49.7.4 \pm 0.4$) in a certain periods than H and NN genotypes. In terms of body weight, H genotype was superior because it has promising growth characters compare to other genotype. Based on the performance of produced generations, the study revealed H genotype to be good starting material for improving meat production. Reproductive performance of Non-descript Deshi chicken is comparatively better than those of other two genotypes and may be used for egg production.

Keywords: generation, genotype, indigenous chicken, selection

S2-0024 The possible involvement of WNT signaling in chicken follicle selection

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In the period of follicle selection, one of the earliest marker for the most recently selected follicle is the elevated expression of FSHR mRNA in granulosa cells. The mechanism of increased FSHR mRNA in singaling small yellow follicle (SY) for selection is not clear. WNTs are a family of secreted glycoproteins that are responsive to FSH signal. The objective of this study is to determine the relationship between FSHR expression and WNT signaling pathway in chicken follicles. We sampled SYs from 3 Jinling Bairi chicken, checked their FSHR mRNA expression levels by qRT-PCR, and performed transcriptome analysis by using Illumina HiSeq 2500 of the SY follicles with the highest and the lowest FSHR mRNA expression. Hundreds of significantly differentially expressed genes were found in 3 groups. We validated the mRNA expression level of some genes by qRT-PCR, and found that the differences of expression level between high-low groups of FSHR expression were the same as RNA-seq. Notably, differentially expressed genes in WNT signaling pathway were revealed. The expression of WNT4, FZD1 and WIF1 were increased accompanying with increase of FSHR mRNA level, but WNT5a and WNT9a were decreased. In different sized follicles, WNT4, FZD1, WIF1 and WNT9a were highly expressed in the SY, but WNT5a was not. Immunofluorescence results showed that, both WNT4 and FZD1 were expressed in the thecal cell of SY follicles. Before SY was selected into hierarchy follicles, they express different levels of FSHR. FSH acts on FSHR in granulosa cells, then it can affect the expression of other genes. The SY with higher FSHR expression is likely selected into hierarchy follicles. In recently report, WNT5a could antagonize gonadotropin responsiveness by suppressing canonical WNT signaling. So we suppose that FSH promote the differentiation of granulosa cells in SY with the activation of WNT signaling pathway and the suppression of noncanonical WNT signaling. We are currently testing this hypothesis.

Keywords: chicken; RNA-seq; WNT4; follicle selection

S2- 0025 Expression dynamics and regulation of PTHLH and its receptor PTH1R genes in chicken follicle development

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Parathyroid hormone-like hormone (PTHLH) was initially identified as the tumor product that is responsible for most instances of humoral hypercalcemia of malignancy. Chicken PTHLH regulates cell growth and differentiation, bone development and lactation, embryonic and fetal development and survival, morphogenesis, and placental calcium transport via intracellular, paracrine and endocrine pathways. The parathyroid hormone (PTH) and PTHLH genes are evolutionarily related and share a common receptor, the PTH/PTHLH receptor (PTH1R). In our previous study, by RNA-seq, we found that the relative mRNA expression of PTHLH is similar to that of FSHR in the single small yellow follicle (SY, 6-8mm). In this study, we examined the expression of PTHLH and its receptor PTH1R in different follicles and the regulation of PTHLH and PTH1R in ovarian theca cells. The results indicated that PTHLH and PTH1R mRNA is expressed at the highest levels within SY follicles, both were expressed in theca cells. For prehierarchy theca cells, a 24h preculture with culture medium followed by a challenge with FSH(5- 100ng/ml) increases PTHLH mRNA expression in a dose-related fashion, and PTH1R expression was also increased by a lower concentration treatment. Interestingly, in the theca cells of posthierarchy follicles, challenge with FSH or E2 reduces the expression of both PTHLH and PTH1R. We further investigated the regulation mechanism of PTHLH transcription by analysing its promoter and identified some negative regulatory elements. Collectively, the present study provides the first evidence of expression and regulation of PTHLH in chicken ovary, and suggests that PTHLH expression in theca cells is modulated by FSH at the time of follicle selection, and likely plays an important role in this process.

Keywords: chicken, PTHLH, PTH1R, follicle selection, theca cells

S2- 0026 Parallel evolution of polydactyly traits in Chinese and European chickens

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Polydactyly is one of the most common hereditary congenital limb malformations in chickens and other vertebrates. The zone of polarizing activity regulatory sequence (ZRS) is critical for the development of polydactyly. The causative mutation of polydactyly in the Silkie chicken has been mapped to the ZRS; however, the causative mutations of other chicken breeds are yet to be established. To understand whether the same mutation decides the polydactyly phenotype in other chicken breeds, we detected the single-nucleotide polymorphism in 26 different chicken breeds, specifically, 24 Chinese indigenous breeds and 2 European breeds. The mutation was found to have fully penetrated chickens with polydactyly in China, indicating that it is causative for polydactyly in Chinese indigenous chickens. In comparison, the mutation showed no association with polydactyly in Houdan chickens, which originate from France, Europe. Based on the different morphology of polydactyly in Chinese and European breeds, we assumed that the trait might be attributable to different genetic foundations. Therefore, we subsequently performed genome-wide association analysis (GWAS) to locate the region associated with polydactyly. As a result, a ~0.39 Mb genomic region on GGA2p was identified. The region contains six candidate genes, with the causative mutation found in Chinese indigenous breeds also being located in this region. Our results demonstrate that polydactyly in chickens from China and Europe is caused by two independent mutation events that are closely located in the chicken genome.

Keywords: chicken, parallel evolution, polydactyly, ZRS, GWAS

S2-0028 The selection of autosex interlinear forms of poultry (chicken, geese, Guinea fowl)

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New autosex forms of poultry (chicken, geese, Guinea fowl) were selected; selection of each species was conducted on 10-20 nests. The presence of sex-linked marker genes was studied. The study of Kuchinskaya Yubileynaya breed (chicken) showed the presence of genes *eb* (basic genes *s+ Co Pg*) and *MI*. The consolidation of chicken based on these genes improved accuracy of sexing from 87 to 94%. In Zagorskaya Lososevaya chicken modifier genes «*ebony*» and *Mh* (basic genes *S co+ ewh*) were revealed. Families with accuracy of sexing 79-82% were identified. News cross of egg-type chicken was selected based upon Red and White Rod Island and White Plymouth Rock, carriers of marker genes *S*, *s*, *K*, *k*. The resulting cross is 3 times autosex: paternal and maternal parental forms can be sexed according to growth rate of feathering, final hybrid according to fuzz color; the accuracy of sexing of day-old chicks is 99.6-100.0%. The study of maternal form of meat-type chicken revealed 5 types according to growth rate of feathering; the subsequent breeding using types 1-4 improved accuracy of sexing by 5.9%. The crossing of Italian geese (*GG Sd/Sd*; *Sp/sp C/C*) with local Shadrinskaya breed (*G/- Sd/-*; *Sp/Sp C/C*) gave few autosex types according to fuzz and feather color. The accuracy of sexing of day-old goslings is 92-94%, at 10 weeks of age 97-98%. The use of marker genes *S* and *s* for selection of parents according to fuzz pigmentation improved accuracy of sexing in day-old Ural White geese and Creme Guinea fowl breeds by 2.7-7.5%. In all 3 species of poultry families with 100% accuracy of sexing were identified. The productivity in newly selected forms is well comparable with modern commercial crosses.

Keywords: chicken, geese, Guinea fowl, genes, autosex

S2- 0029 Heart transcriptome response of fast- versus slow-growing broilers under heat stress

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Modern broilers with rapid growth and large body size are highly susceptible to heart failure due to imbalanced development of hearts relative to muscle. When exposed to high temperature, broilers have increased mortality due to cardiovascular dysfunction. To improve cardiac function of modern broilers under heat stress through breeding, we must identify significant genes and critical pathways that are responsible for susceptibility to heat stress. We compared cardiac transcriptome response to heat stress in a modern broiler line with fast growth rate (Ross 708) and a legacy broiler line with slow growth rate (Illinois). After heat stress treatment at 39°C for 8 hours/day from 21 days to 42 days posthatch, only the Ross broilers showed reduced normalized heart weight compared to the control-temperature group at the same age. In RNAseq study of left ventricles, 325 differentially expressed (DE) genes were found between the heat stress and control group of Ross broilers, but only 3 DE genes were found in Illinois broilers. Ingenuity pathway analysis indicates significant downregulation ($P < 0.05$) of multiple regulator effects involved in cell proliferation, cell movement, angiogenesis and quantity of immune cells, and up-regulation of regulator effects involved in cardiac hypertrophy, heart contraction and heart rate in Ross broilers under heat stress compared to control temperature. In addition, the comparisons between the two broiler lines indicates that cell cycle activity is higher in Ross than in Illinois birds under normal temperature, but lower in Ross than in Illinois under heat stress. Among the top ranked canonical pathways, mitotic role of polo-like kinase pathway is the most significant one in all comparisons. This result indicates that the increased susceptibility to heart failure of modern broilers under heat stress may be due to impaired heart development induced by retarded cell cycle activity, thus revealing a potential target for genetic improvement.

Keywords: heat stress, broiler, heart, RNAseq, cell cycle.

S2-0030 Genomic selection in a nuclear breeding population of layers

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Genomic selection has revolutionized the animal breeding industry and it's becoming a new method for selecting the best sires in cattle in place of progeny testing, because of its accuracy and shorter generation interval. In other species, such as poultry and pigs, selection can also benefit from genomic selection if the selection is conducted in the nuclear population. Here, we explored the feasibility of genomic selection in a nuclear breeding population in China. A total of 1341 chickens from 4 generations were genotyped with a 600K high density SNP chip. Egg weight at 28 week (EW28), laying rate at 38 week (LR38), eggshell strength at 38 week (ESS38), and albumin height at 36 week (AH36), were analyzed. The reliabilities of conventional estimated breeding values for these traits are 0.614, 0.281, 0.392, and 0.410, on average, respectively. Two scenarios were studied to validate the genomic prediction models. The first is five-fold cross validation using 1146 females of generation 4, in which genomic best linear unbiased prediction (GBLUP) model was used. The predictive ability was calculated as the correlation between genomic estimated breeding values (GEBV) with traditional estimated breeding values (EBV). The predictive abilities were in the range of 0.590 to 0.734. In the second scenario, we used males from generation 1 to generation 3 as training set to predict the young males in generation 4. The models employed are GBLUP, and two single step methods, i.e. single step GBLUP (ssGBLUP) and single step blending (ssBlending). The latter two integrate pedigree relationship and genomic relationship, thus can absorb non-genotyped animals, and are expected to yield improved predictive ability. On average, ssGBLUP and ssBlending outperforms GBLUP by 18% and 7%, respectively. It was concluded that it is promising to adopt genomic selection in nuclear populations of layers and single step genomic selection methods yields better predictive ability than GBLUP method.

Keywords: genomic selection, layers, nuclear, GBLUP, single step

S2-0031 Copy- number polymorphism of the SOCS2 gene in diversified chickens and its relationship with body size

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It has been reported that SOCS2 has dual effects on growth hormone (GH) signaling depending on dosage: both non-expression and overexpression of SOCS2 would lead to higher sensitivity of cells to GH action. Previously, we found SOCS2 with high copy number (CN) in Luxi Game but not in other chickens by next generation sequencing. There is a strong implication that the highly duplicated SOCS2 may be related to larger and stronger body. To validate the copy number polymorphism of the SOCS2 gene and its relationship with body size, we quantified the copy number value of diversified chickens by qPCR. Chickens included four cockfights, i.e. Luxi Game(LX), Zhangzhou Game(ZZ), Tulufan Game(TLF), Henan Game(HN), and five other breeds, i.e. ShouGuang (SG), Cobb500 (parental line), White Leghorn (WL), Rhode Island Red (RIR). The results demonstrated that the average CN values of SOCS2 were highly variable among diversified chickens and the highest one was found in LX (CN=5.0). The CN estimates of all kinds of cockfights were significantly larger than that in other breeds. As cockfights were known for larger and stronger body, we deduced the conclusion that their big body size may be related to high CN in SOCS2. Our results also suggested that copy number of SOCS2 gene was a common variation in the autosomal region.

Keywords: SOCS2, copy number, body size

S2-0032 Genome-wide detection of selective signature in chicken

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The chicken have experienced dramatic selection due to domestication, which has led to many different phenotypes for multi-purpose. Selection signatures are the selective footprints across the organism genome due to the effect of artificial selection, which displayed the long range linkage disequilibrium in chromosome or genetic diversity reduction. Currently, genome-wide scans in both cattle and humans showing positive selection footprints have been investigated. However, few studies have focused on a genome-wide scale in chicken. Here, we employ 600 K high density Affymetrix Axiom Chicken Genotyping Array with the method of EHH (Extended Haplotype Homozygosity) test to detect genome-wide selective signatures of the F2 population, which was developed by reciprocal crosses between White Leghorn and Dongxiang chicken. A subset of the putative regions showing the highest significance in the genome-wide EHH tests was mapped. We annotated genes to identify possible influence they have in beneficial traits by using the functional gene set enrichment analysis. A panel of genes, including AASDHPPT, PAR3, Sox6 and AKT Signal pathway were detected, which overlapped with the most extreme P-values. This panel comprises some interesting candidate genes and QTL, representing immune function and neurogenesis. The results of this study provide a genome-wide map of selection signature in the chicken genome, and can be valuable for revealing the genetic mechanisms of phenotypic diversity in poultry breeding.

Keywords: selective signature, chicken, EHH, candidate gene

S2-0033 Gga-miR-219b and its target BCL11B gene were involved in chicken Marek's disease lymphoma transformation

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Marek's disease (MD) is a kind of contagious disease that induces lymphomas in poultry caused by Marek's disease virus (MDV). MicroRNA (miRNA) is important in regulating tumorigenesis. It has reported that miRNA was involved in MD. Our previous study showed gga-miR-219b was down-regulated in MDV-induced lymphoma and its target gene BCL11B was predicted. We verified BCL11B was the target of this miRNA through RT-qPCR, Western blotting and that this miRNA was involved in lymphoma formation through CCK-8 assay, flow cytometry and RNA interference. BCL11B gene was increased in MD lymphoma than that in non-infected spleens. We confirmed BCL11B was the target of gga-miR-219b through dual-luciferase reporter assay. The results of RT-qPCR showed that BCL11B was down-regulated in agomir transfection group at 24h, 48h and 72h while it was up-regulated in antagomir transfection group at 48h and 72h. Interestingly, MDV oncogene Meq was down-regulated in agomir transfection group at 48h and up-regulated in antagomir transfection group. We found that gga-miR-219b could inhibit cell proliferation by promoting cell apoptosis not by regulating cell cycle through CCK-8 assay and flow cytometry. We also used siRNA to interfere with BCL11B to see whether it could influence function of tumorous cells. Expectedly, siRNA-BCL11B could interfere with BCL11B at up to 90% interference efficiency. When BCL11B was knocked down in MSB1 cells, proliferation was greatly inhibited at 24h, 36h, 48h, 60h and 72h and apoptosis was increased at 48h after transfection, but it didn't affect cell cycle. Meanwhile, migration of MSB1 cells was greatly inhibited. The results concluded that both gga-miR-219b and BCL11B might play important roles in MD lymphoma transformation.

Keywords: Marek's disease, BCL11B, gga-miR-219b, RNA interference

S2-0034 Hypothalamus gene expression profiling in SPF White Leghorn inoculated with the *Campylobacter jejuni*

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Campylobacter jejuni (*C. jejuni*) is a food-borne pathogen, which can cause great threat to human health through the contaminated poultry products. Chicken is the main reservoir of the *C. jejuni*. Bacteria in the gastrointestinal tract influences behavior and brain function. It has been reported that hypothalamus-pituitary-thyroid interacts with immune system. It is worth to know the transcriptome in hypothalamus in the response to *C. jejuni* inoculation. To study the transcriptome in hypothalamus following *C. jejuni* inoculation, 28 *C. jejuni* free 3-day old SPF white leghorn chickens were orally inoculated with 500 μ l inoculants of 1×10^8 cfu/chicken as inoculated group, 28 were mock inoculated with 0.5 ml sterile PBS as non-inoculated group. Four birds in each group were randomly selected and sacrificed by cervical dislocation at 8 hours post inoculation. The hypothalamus were collected for RNA extraction. Three individual samples in each group were used for transcriptome analysis through next generation sequencing. The results showed that 19,061 genes were expressed in either group with 16,312 known genes and 2,749 new genes. There were 102 genes only expressed in non-inoculated group. There were 135 genes were significantly differentially expressed between inoculated and non-inoculated groups. The differentially expressed genes were submitted into DAVID for GO and Pathway enrichment analysis. There were 417 GO biological process terms enriched including ($P < 0.05$), such as immune system process and immune effector process. The Hedgehog signaling pathway, the Jak-STAT signaling pathway, the Wnt signaling pathway, and the Cytokine receptor interaction were enriched following *C. jejuni* inoculation. The results herein will provide a crucial theoretical foundation to understand the molecular mechanism of response to *C. jejuni* inoculation in chicken.

Keywords: transcriptome, hypothalamus, SPF chicken, *C. jejuni*

S2- 0035 Dynamic expression of HSP90 mRNA in the hypothalamus and SNP detection in the gene: marker for heat tolerance in Chinese chicken breed

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Heat stress is one of the main limiting factors in poultry production due to its adverse effects on feed intake, growth rate, egg production, hatchability and mortality of birds. This study investigated the effects of heat stress on the expression of HSP90 mRNA in the hypothalamus and detected HSP90 variations in chicken. Females of two Chinese chicken breeds, Huainan chickens ($n=200$) and Wenchang chickens ($n=200$), were used for the experiments. On 64 days of age, the ambient temperature ($24 \pm 1^\circ\text{C}$) was increased to $35 \pm 1^\circ\text{C}$ for 24 hours (heat stress), then decreased to $24 \pm 1^\circ\text{C}$ (recovery). Hypothalamus samples were collected at 0, 12, and 24 hr during heat stress, as well as 12 and 24 hr during recovery. Expression of HSP90 mRNA increased significantly during heat stress and significantly decreased during recovery; expression was higher in Huainan chickens. Fifteen primers were designed to amplify the exons of HSP90 by PCR, and SNPs were detected by sequencing. Females of Huainan chickens ($n=117$) at 65 days of age were used for polymorphism tests, the ambient temperature ($24 \pm 1^\circ\text{C}$) was quickly increased and maintained at $42 \pm 2^\circ\text{C}$. We identified a SNP (A1570G) in exon 4 of HSP90 which did not change the amino acid sequence; the genotypic frequencies of AA, GA, and GG were 0.49, 0.27, and 0.24, respectively. Individuals with the GG genotype survived heat stress at 42°C for 248.2 ± 39.3 min, compared to individuals with GA and AA genotypes, which survived for 227.2 ± 44.5 min and 179.3 ± 36.5 min, respectively.

Keywords: chicken, heat stress, HSP90, mRNA expression, SNP

S2-0037 The selecting experiment of resistance to duck hepatitis virus of ducks

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The infected experiment of two generations of Peking ducks was finished in the way of inoculating two generations with DHAV-3 virus followed by analyzing the mortality and comparing the detection rate of F1 generation. The F1 generation was divided into five groups according to the resistance of parental generation. The parents of A group were the same with the F0 generation who has not been inoculated with DHAV-3 virus ever. The parents of B1 group were those whose family mortality to DHAV-3 virus was from 0 to 10%, meanwhile the parents of B2 group were those left survivors. The parents of C1 group were the uninoculated full sibs of group B1, while the parents of C2 group were the unvaccinated full sibs of those whose family mortality was from 90% to 100%. The results showed that: (1) the parents of F0 generation have 88 families (full sibs ≥ 7), in which there are 9 families whose mortality is 0% and 14 families whose mortality is 100%. (2) There are 119 families in B1 and B2 group, while the amount of families whose mortality is 0% is 104. The mortality of these two groups is 5.72%, which is significantly lower than that of F0 selected experimental group. There are 17 families in C1 and C2, in which there are 15 families whose mortality is more than 59.04%. The mortality of these two groups is 70.7%, which is significantly higher than F0 generation. (3) The higher mortality of F1 generation group, the higher detection of DHAV-3 in its cloacal swab. The results showed that the resistance to DHAV-3 virus could be improved by genetic selection, which could provide valid theory basis for the further large scale of breeding of ducks.

Keywords: duck, F0 generation, F1 generation, mortality

S2- 0038 Genetic parameters for growth and carcass traits of Peking ducks

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In Peking ducks there is a remarkable breeding progress. From a heavy strain pedigreed birds could be used for calculation of heritability as well as phenotypic and genetic correlations. The ducklings were kept from the age of 15 to 42 days in single boxes with individual feeding of standard duck grower pellets. Beside of growth it could be measured feed efficiency and breast muscle thickness. All 6 weeks old ducks were slaughtered, the carcass composition was measured. The genetic parameters were estimated by software packages PEST (GROENEVELD et al., 1990) and VCE-6 (GROENEVELD et al. 2008). The average body weight (42nd day) was 3.72 kg with $h^2 = 0.47$. The feed efficiency from 15 to 42 days of age is 0.45. The heritability for feed efficiency is $h^2 = 0.36$. The breast muscle thickness amounts to 1.98 cm with $h^2 = 0.56$. The same heritability was estimated for breast muscle percentage. The genetic correlation between breast muscle thickness and the percentage of breast muscle to carcass with 0.78 is high enough for practical selection. The genetic correlations of feed efficiency to breast muscle thickness and to breast muscle percentage are low with 0.04 and 0.17, respectively, but is high to leg muscle percentage ($rg = 0.66$). There is also a high negative genetic correlation between feed efficiency and amount of skin with $rg = -0.53$. It means the higher the feed efficiency the lower the amount of skin with subcutaneous fat. High feed efficiency is a presupposition for optimum biological efficiency and sustainability in duck production. The objective of successful duck breeding for meat production is of the percentage of breast muscle and the feed efficiency.

Keywords: body weight, feed efficiency, breast muscle thickness, muscle percentage, genetic and phenotypic correlations, heritability

S2-0039 Gene expression profiles of nerve growth factor and its receptors in green eggshell chickens during different laying periods and across tissues

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Nerve growth factor (NGF) play an important role in regulating reproductive systems including sexual development, follicular development and ovulation through autocrine or paracrine pathways collaborating with reproductive hormones and other cytokines. The aim of this study was to investigate the expression of NGF and its receptors tyrosine kinase A (TrkA) and p75 in different tissues of green eggshell chickens at different physiological stages and four ages (19, 33, 50 and 64 weeks). The NGF and its receptors were highly expressed in ovarian tissues, and the expression of NGF in hierarchical follicles F1-F4 was greatest at 19W and 33W. In prehierarchical follicles, NGF and TrkA expression was greater in early than later periods of follicle development at 19W, 33W and 50W. The expression of NGF in all ovarian follicles was greater at 33 weeks than other ages. The expression of NGF during the last stage of laying was lowest as compared to other stages. In ovarian tissue, the expression of TrkA was greatest in all examined tissues at each physiological stage during the last stage of laying. In contrast, the expression of p75 was lowest during this stage. In the hypothalamus, the isthmus, and LWF the expression of NGF was greater during the late laying period than the brooding period. Our results show that the expression of NGF and its receptors in chicken tissues exhibit specific developmental changes and age-related patterns and revealed a close correlation between NGF expression and follicular development, which suggests that NGF ligands and receptors are likely involved in the regulation of cyclic changes in the ovarian function of birds.

Keywords: NGF, TrkA, p75, chicken, mRNA expression

S2- 0040 Variants and methylation level in the promoter regulate the expression of Cystathionine Beta Synthase in laying hens

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Bone fragility is common in end-of-lay hens due to the attrition of structural bone during egg production. The improvement of bone quality will benefit for the welfare of the laying hen and the sustainability of egg production. Cystathionine Beta Synthase (CBS) is rate-limiting enzyme in one carbon metabolism. The expression and activity of this enzyme is thought critical for maintaining normal bone. The aim of the research is to understand the mechanism for regulating transcriptional expression of CBS gene in the bone of laying hens. Methods: Reporter constructs with different flanking sequences from the CBS gene were made to identifying the core promoter region. Evaluation of the effects of these constructs and variants in the upstream CpG Island on gene expression was performed using a dual luciferase detection system. The region containing the variants was used for methylation analysis with bisulfite sequencing. CBS gene expression level was measured by QRT-PCR. There was a large difference in expression of CBS mRNA ($1.6\text{e-}12$ to $192\text{e-}12$ moles) in the bones of the laying hens. We identified the core promoter region of the CBS gene in chicken and a number of variants. A 7bp and 18bp/12bp insertion/deletion constituting three alleles was detected in the CpG island. The promoter activity of the recombinant with allele 1 (7bp deletion and 12bp insertion) was significantly lower than that of allele 2 (7bp insertion and 18bp deletion) and allele 3 (7bp deletion and 18bp insertion) ($P < 0.01$). The results indicated that variants in the gene sequence were associated with gene expression. The methylation status in the 18bp/12bp insertion sequence was also related with gene expression. Therefore, the deletion/insertion variants and methylation sites in the CpG island may be responsible for the differing expression level of the CBS gene in the bone of laying hens.

Keywords: CpG island, promoter, Cystathionine Beta Synthase, hen

S2-0041 An effective GBS protocol for SNP discovery and genotyping in Peking ducks

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Domestic duck is an economically important agriculture animal and is consumed worldwide, especially in Asia. Meanwhile, duck also is a suitable material for population genetics and evolution studies. However, there is a limitation for population genetics study and genomic selection due to lack of duck-specific DNA chip platform. Here, we developed an effective and low-cost genotyping-by-sequencing (GBS) protocol for ducks and genotyped 49 Peking ducks for performance assessment. All the samples' genomic DNA were extracted from blood using the standard phenol/chloroform method. According to the results of simulated digestion, genomic DNA was digested with restriction endonuclease, *MseI*, and then to be used in library construction. The data of 2×125 bp pair-end reads was generated by the Illumina HiSeq2500. After quality control, clean reads were aligned to the duck reference genome (BGI 1.0, Ensemble 82) using Burrows-Wheeler Aligner (BWA) with the default parameters. SNP calling and genotyping were performed by Genome analysis toolkit (GATK). As a result, a total of 169,010 SNPs were identified from all animals and a mean of 55,920 SNPs were obtained for each individual. The average of SNP density reached 70 SNP/MB. In this study, GBS was first performed on domestic duck. We found that GBS is a highly-effective method for genome-wide genotyping with accessibility and low cost-performance in ducks.

Keywords: domestic ducks, reduced-representation genome, diversity

S2-0042 Analysis of duck genome re-sequencing data reveals selection patterns during duck domestication and speciation

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To identify the selected regions and genes during duck domestication and speciation, we carried out duck genome resequencing of five populations representing three domestic duck populations (a lean line of Beijing duck (ZF), a fatness line of Beijing duck (ZM) and China Micro-duck (CMD)), an ancestor population (mallard, M) and a wild duck population captured nearby seaside of East China sea (W). Thirty individuals of each population were blooded, extracted DNA for each of them, mixed with equal Moore, constructed a DNA library, and sequencing for $\sim 40\times$ depth. In total, 17,792,975 single nucleotide polymorphisms (snps) and 948,725 insert and deletion (Indels) were found and most of the mutations (snps and indels) were silent mutations or modifiers. We also found 133 selective sweeps occurred in all domesticated ducks covering 78 selected genes and 134 selective sweeps between ZF and ZM representing 99 related genes respectively. The GO results of the relevant genes above showed that the most important selected traits for domesticated ducks were feather color and substance synthesis and that for speciation duck were fat deposition and muscle development. We found evidence that feather color were strongly selected in domesticated ducks because of there were some important mutations occurred in pigmentation-related genes *MITF* and *LYST*. Also, we found that 16 genes out of the 99 speciation-related genes were relevant to skeletal muscle development and fat deposit and that the protein of *IGF2R*, a negative regulator for muscle mass, were disturbed by a nonsynonymous substitutions at Trp1837* in ZM which resulted in a functional loss in ZM. This study provides a genome-enabled improvement in duck breeding.

Keywords: duck, resequencing, selection patterns, domestication, speciation

S2- 0043 Transcriptome analysis of ODC and OAZ gene expression in pre-hierarchical follicles of geese

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The ornithine decarboxylase (ODC) and ornithine decarboxylase antizyme (OAZ) regulate animal reproduction by control polyamine formation. The geese follicular development is a very complicated process. To research the enzymes expression in pre-hierarchical follicles of geese, we selected Jilin Zi geese as the study objects. We performed de novo assembly to investigate 5 stages of pre-hierarchical follicles of geese through Illumina HiSeq 2500 platform. The results show that: The ODC and OAZ genes are expressed in all pre-hierarchical follicles of geese, and there is difference in tissue. The expression of ODC expression with the follicular development decreased at first, then increased, and decreased finally. The OAZ expression with the follicular development increased at first, and then decreased. Through analysis the results we found that the expression of ODC gene in the middle white follicles is the lowest and the expression in the primary follicular is the highest. And there is no significant difference with others ($P>0.05$). The OAZ gene expression in the middle white follicular is the highest. The expression of OAZ in the small yellow follicular is the lowest and shows significantly lower than other groups ($P<0.05$). After analysing the relativity, the expression of ODC in 5 stages of pre-hierarchical follicles shows negative correlation with the expression of OAZ.

Keywords: ODC, OAZ, geese, pre-hierarchical follicles, transcriptome

S2- 0044 Characterization of Aseel chicken for morphological, reproductive, growth and production parameters up to 64 weeks of age

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Aseel bird is an important native chicken breed known for its martial qualities, pugnacity, majestic gait and agile. The aim of the study is to conserve the Aseel germplasm which is considered to be endangered under in situ conditions. The birds were maintained at Directorate of Poultry Research, Hyderabad on deep litter under a simulated backyard type of housing (having night shelter and open range). A total of 236 chicks produced randomly from parents collected from the native tract in the state of Andhra Pradesh, India in 2nd generation were characterized for physical, morphological, growth and production parameters with standard procedures. Aseel birds are characterized by multi coloured plumage (predominantly dark brown, black, golden etc.) with solid feather pattern and normal distribution. The long glossy tail feathers add to the beauty of the bird. Ear lobes were red (92%). All the birds had red coloured pea combs with variations in intensity of colour and size. The shank colour was yellow in majority (88%) of the birds. Spur was observed in majority of the birds with varying sizes (96%). The fertility and hatchability was 67.18 and 41.4% respectively. The body weight at 0 day, 4, 6, 12 and 16 weeks of age was 28.93 ± 1.1 , 142.39 ± 5.40 , 297.71 ± 6.41 , 821.22 ± 10.15 and 1302.09 ± 12.15 g, respectively with the corresponding shank length of 43.53 ± 0.94 , 57.87 ± 1.24 , 92.50 ± 1.81 , 112.02 ± 1.98 mm. Cocks were heavier with distinct sexual dimorphism in Aseel. The body weight at 20 and 40 weeks was 1548.1 ± 18.15 and 1904.9 ± 33.30 g in hens and 2348.8 ± 22.17 and 3148.5 ± 47.52 g in cocks, respectively. The age at sexual maturity was 214.13 ± 5.92 days. The part period egg production up to 40, 52 and 64 weeks of age was 18.20 ± 1.24 ($n=88$), 30.07 ± 1.99 ($n=75$) and 41.44 ± 3.44 ($n=65$), respectively. The egg weight at 40 weeks of age was 40.27 ± 0.42 g. Efforts are on to improve the productivity of Aseel chickens without compromising on the original breed characteristics.

Keywords: Aseel, growth, egg production, shank length

S2-0045 Multi-Trait Genome-wide association studies identifies genetic variants and candidate genes for longitudinal body weights in chicken

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This study aimed at revealing the genetic architecture underlying the dynamic body weight of hens. Here, we constructed the experiment population of 1,534 F2 hens derived from reciprocal crosses between White Leghorn (WL) and Dongxiang chickens (DX). We measured the body weight (BW) at sixteen time points from hatching to 20 week of age, average daily gains (ADG) from fortnightly body weight, and body length (BL) and keel length (KL) at 13 week of age. The best nonlinear model was chosen to describe the weight-age data, and the growth inflection point was used to divide the growth phase. In addition, we conducted univariate and multivariate phenotypes GWAS analysis utilizing 600 K SNP array in an F2 chicken population with longitudinal body weights. We identified a narrow 0.8 Mb region spanning from 75.4 to 76.2 Mb of chromosome 4 (GGA4) to be strongly associated with chicken late growth during weeks 8-20. A total of nine annotated candidate genes had interesting biological functions related to muscle development, skeletal muscle development, bone development. The rs318027552 (GGA1) with a candidate gene ATP7B was found to be significantly associated with all body weight traits except BW0 and accounted for 4.61~17.45% of the phenotypic variance for BW from 1 to 20 week of age. Our study was the first analysis of longitudinal body weight data using multiple phenotypes. Identification of the promising region as well as potential candidate genes could be helpful to future marker-assisted selection and genomic selection in chickens.

Keywords: GWAS, longitudinal body weights, candidate gene, chicken

S2-0046 Genetic diversity of Prolactin gene in Japanese quail (*Cortunix cortunix japonica*) in Nigeria

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Poultry production has been documented to be a proven strategy to alleviate poverty in the developing world. Among poultry species, quail is the easiest to produce, yet no proper breeding strategy exist in Nigeria. However, data on production characteristics and genetic diversity among quail populations in Nigeria is scant. This study was carried out to investigate the genetic diversity, relationship and population structure in two Japanese quail strains (Albino and wild) using a restricted fragment length polymorphism (RFLP) marker in the prolactin (PRL) gene. Fifteen quail from each strain were sampled in 5 geographical regions in Nigeria (Kano, Jos, Umudike, Port Harcourt and Ibadan). Polymerase chain reaction (PCR) and electrophoresis was used to characterize a 24 base pair (bp) insertion/deletion in a 358 bp PCR product. The populations were analysed for their genetic variability using allele frequency, molecular variance, deviation from Hardy-Weinberg (H-W) equilibrium using the phylogenies package (PHYLIP) and Analysis of molecular variance (AMOVA). The frequency of insertion (A allele) was similar for both strains in the Ibadan, Jos and Umudike populations, however, the allele frequency was 0.73 and 0.50 for the Albino and Wild strains, respectively in Kano, and 0.57 and 0.70, respectively for the Albino and Wild strains in Port Harcourt. Whereas there were no deviations from Hardy-Weinberg equilibrium for both strains, in Ibadan, Jos and Umudike, the populations in Kano and Port Harcourt deviated from H-W equilibrium. AMOVA analysis showed that 3% of the total genetic variation was explained by population difference, 19% by variation among individuals and 77% within individuals. Prolactin is an important gene for reproduction, and segregation of the RFLP marker could be assessed for reproductive capacity. The delineation of genetic diversity in these populations allows for innovative selective breeding and conservation strategies to be developed.

Keywords: prolactin, diversity, Japanese quail, conservation, selection

S2- 0047 Genome- wide association studies for comb traits in chickens

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The comb, as a secondary sexual character, is an important trait in chicken. Indicators of comb length (CL), comb height (CH), comb weight (CW), and comb size (CS) are often selected in production. The CS shows positive correlation with bone density and egg production. DNA-based marker-assisted selection could help chicken breeders to accelerate genetic improvement for comb or related economic characters by early selection. Although a number of quantitative trait loci and candidate genes have been identified with advances in molecular genetics, candidate genes underlying comb traits are limited. The aim of the study was to use genome-wide association (GWA) studies by 600 K Affymetrix chicken SNP arrays to detect genes that are related to comb, using an F2 resource population. For all comb characters, comb exhibited high SNP- based heritability estimates (0.61~0.69). Chromosome 1 explained 20.80% genetic variance, while chromosome 4 explained 6.89%. Independent univariate genome- wide screens for each character identified 127, 197, 368, and 205 novel significant SNPs with CL, CH, CW, and CS, respectively. Three candidate genes such as VPS36, AR and WNT11B were determined for having a plausible function in all comb characters. These genes are important to the initiation of follicle development, gonadal growth, and dermal development, respectively. The current study provides the first GWA analysis for comb traits. Identification of genetic basis as well as promising candidate genes will help us understand the underlying genetic architecture of comb development and has practical significance in breeding programs for the selection of comb as an index for sexual maturity or reproduction.

Keywords: genome- weide association studies, chicken, comb, androgen receptor

S2- 0048 Association analysis between genome- wide copy number variation and egg number using chicken 600 SNP chip

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Egg number is important to the egg production and producers' profit. Copy number variations (CNVs) are currently accepted as a common source of genetic variation. To investigate CNVs for egg number traits, association analysis was performed using the chicken 600 K genotyping array. Egg number, including laying periods from 21 to 26 weeks (EN1), 27 to 36 weeks (EN2) and 37 to 72 weeks (EN3), were collected for 1,534 F2 hens derived from reciprocal cross between White Leghorn and Dongxiang Blue-shelled (DBS) chicken as reported previously. For each trait, phenotypic values that did not fall into the range of [mean \pm 3 standard deviations (SDs)] were removed prior to the analysis. Chicken genomic DNA was isolated from whole blood samples and genotyped using the chicken 600 K SNP chips. Quality control was performed using the APT package with standard protocol of chicken. PennCNV program was applied to the CNV identification with autosomes for 1,512 eligible hens. CNVRs were determined by aggregating overlapping CNVs identified across samples. Gene content of chicken CNV regions was assessed using Ensembl genes. A total of 8,728 CNVs in 1,512 samples was detected. After overlapping, 953 CNVRs were obtained across 30 autosomes, in which the most frequent CNVRs was spanned a region from 72.86 Mb to 72.91 Mb on chromosome 1 with 28 SNPs harboring in it, of which 4 SNPs were located in the intron 1 of fatty acyl CoA reductase 2 (FAR2). The CNV was shared by 57 individuals, in which five hens with 0 copy number (CN0), and 52 hens with 1 copy number (CN1). Student test showed that EN2 (peak laying period) has a significant difference ($P < 0.001$) between CN0 and CN1. Moreover, average egg number in CN0 is 56.29, and the average egg number in CN1 group is 60.40. In the present work, we detected a CNVR and a gene FAR2 as candidate for EN2, which need a further investigation and might be applied in the chicken breeding.

Keywords: copy number variation, egg number, association analysis, chicken

S2- 0049 Identify the promising genes for the chicken oviduct uterus by genome-wide association studies

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Egg production and quality, as economic traits, play determine role in poultry industry. Improving the egg number to 500 is the ultimate goal in recent years, while as the layer hen grows old, the eggshell quality and Haugh unit sharply decrease, which is a contradiction between the egg number and egg quality. The oviduct uterus that involve in the protein secretion and shell formation plays a key role in the egg quality. The aim of this investigation was to detect genes or genomic region that are related to oviduct with genome-wide association (GWA) studies by 600 K Affymetrix chicken SNP arrays, using an F2 resource population. The SNP- based heritability estimates for oviduct uterus length and weight exhibited moderate(0.35, 0.39). Chromosome 1 explained 9.45% genetic variance, while from chromosome 4 to 8 and chromosome 11 explained more than 1%. Independent univariate genome-wide screens for oviduct uterus length and weight detected significant SNPs were 82 and 52. The significant genomic region for oviduct weight were ranged from 167.79-174.29Mb on GGA1, 73.16-75.70Mb on GGA4, and 4.88-4.92Mb on GGA8. The list candidate genes are CAB39L, NCAPG, TGFBR1 and GORAB that were identified for have a potential role in oviduct uterus. These genes involvement in mTOR signaling pathway, body weight, cellular processes and secretory pathway, respectively. The results in our investigation was the first GWA analysis for oviduct uterus length and weight. From the results obtained in the experiment, the promising loci as well as potential candidate genes will greatly supplement that the genetic basis underlying oviduct uterus and could have impact on the selection of egg quality.

Keywords: chicken, genome- wide association studied, oviduct uterus, egg quality

S2-0052 The investigation of expression differences of PDGF and its receptor genes in MAKP pathway of prehierarchical follicles in geese

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Mitogen-activated protein kinase (MAPK) is an important signal transduction pathway in organisms, which is involved in mediating processes such as the growth, proliferation, differentiation and so on. Among the signal pathway, Platelet-Derived Growth Factor (PDGF) and its receptor (PDGFR) are involved in regulating the development of ovarian follicles of domestic poultry. The aim of this study is to investigate the expression differences of PDGF and its receptor genes in MAKP signal pathway of prehierarchical follicles in geese. 35-37 weeks-old postnatal female geese were utilized as experimental materials, and Real-time PCR technology was applied to detect the expression differences (divided into 5 grade: SY, L, M, S, Pe) of PDGF and PDGFR genes in prehierarchical follicles in geese. The PDGF demonstrated the highest expression level in SY, and the differences were significant when comparing with L, M, S, Pe ($P < 0.05$). This suggested that PDGF existed in the middle phase of follicular development, and it was involved in the whole series of growth at the late phase of follicles. The expression level of PDGFR was the highest in SY, and the differences between L and M as well as M and S were not significant ($P > 0.05$). Wherein Pe demonstrated the lowest expression level, which showed significant differences when comparing with SY, L, M, and S ($P < 0.01$). This suggested that the hierarchical expression of receptor gene Pe is not the main position for promoting follicular development. This result indicated that PDGF and its receptor genes were expressed in all 5 prehierarchical follicles, and SY is the main expression position, which promotes follicular development by promoting cell proliferation.

Keywords: PDGF, PDGFR, expression differences, follicular development

S2- 0053 The Expression difference of HS6ST2 gene between layer and broiler SMSCs and the preliminary research about the function of HS6ST2 gene

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The HS6ST2 gene belong to the HS6ST gene family. HS6ST2 gene mainly regulate the physiological and pathological processes by changing the sulfate pattern of the heparan sulfate proteoglycan's heparan sulfate chains. Previous studies have provide the evidence of HS6ST2 involved in the regulation of proliferation and differentiation of satellite cells. To further explore the function of HS6ST2 gene in myogenesis, we investigated the expression difference of HS6ST2 gene between broilers and hens satellite cells at different stages of skeletal muscle development. By constructed and transfect the eukaryotic expression vector and shRNA vector of chicken HS6ST2 gene, to 7 days broiler's primary satellite cells, we investigate the function of HS6ST2 gene in satellite cells. Then, we use the real time PCR technology to detect the changes of mRNA expression level of related genes which belong to Wnt/ β -catenin, TGF β /MSTN and Notch signal pathways after the interference or overexpression. After a series research, we draw the following conclusions: (1) The hens primary satellite cell's HS6ST2 gene mRNA expression level is significant higher than broiler at each skeletal muscle development stages during cultured in vitro; (2) Interference or overexpression the HS6ST2 gene in chicken primary satellite cells indeed affected the proliferation and differentiation contion of primary satellite cells and interference the HS6ST2 gene would weaken the satellite cell's proliferation activity, but enchanced its differentiation ability and myotube production; however overexpression the HS6ST2 gene significantly inhibited satellite cell's differentiation, but promoted its proliferation ability; (3) In satellite cell, HS6ST2 gene will boost the TGF β /MSTN and Notch signal pathway' signal transduction, and repress the Wnt/ β -catenin signal.

Keywords: hens, broiler, satellite cell, HS6ST2 gene, proliferation, differentiation

S2- 0054 Expression profiles of the PPP3CA and PPP3R1 genes in phenotypically distinct skeletal muscles of two chicken breeds with different growth rate

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Protein phosphatase 3 (PPP3) is implicated in the control of skeletal muscle fiber phenotype and hypertrophy depending on muscle phenotype and stage of myofiber growth, and the differential expression of the components of PPP3 signaling may contribute to the differences among muscles. However, little information is available concerning the expression of PPP3 in chickens. In the present study, the expression of two PPP3 subunit genes (PPP3CA and PPP3R1 gene) was quantified by RT-PCR for the first time in the lateral gastrocnemius (LG, mainly composing of red fast-twitch myofibers), the soleus (mainly composing of red slow-twitch myofibers) and the extensor digitorum longus (EDL, mainly composing of white fast-twitch myofibers) from Qingyuan partridge chickens (QY, slow-growing chicken breed) and Recessive White chickens (RW, fast-growing chicken breed) of different weeks of ages (0, 1, 3, 5, 7, 9). Although the expression levels of two genes were variable with different trends in different skeletal muscles in two breeds of chicken during postnatal growth, they are highly muscle phenotype and breed specific. In general, the levels of PPP3CA and PPP3R1 gene expression of soleus were lower than those of EDL and LG in both chicken breeds at the same stages. Compared between the two chicken breeds, the levels of PPP3CA gene expression of three skeletal muscles in QY chickens were higher than those in RW chickens at 0 and 3 weeks of age. However, the levels of both PPP3CA and PPP3R1 gene expression of three skeletal muscles were lower in QY chickens than those in RW chickens at 9 weeks of age. Correlation analysis of the levels of PPP3CA and PPP3R1 gene expression of the same skeletal muscle showed that there were positive correlations for all three skeletal muscle tissues in two chicken breeds. These results provide some valuable clues to understand the role of PPP3 in the development of chicken skeletal muscles, with a function that may be related to meat quality.

Keywords: protein phosphatase 3, chicken, skeletal muscles, expression

S2- 0055 Endothelin 3 gene copy number detection and breeding application in Dongxiang black-boned chicken

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Intense pigmentation of skin and internal tissues (fibromelanosis (FM)) is characteristic of black-boned chicken. The dominant FM allele is located between 10.3 and 13.1 Mb on chromosome 20, which has two regions of copy number variation. One region contains the endothelin 3 (EDN3) gene involved in promoting melanoblast proliferation. Dongxiang black-boned chicken is the native breed of Jiangxi province, China. The main skin color among populations is black and gray; about 6% of individuals have white skin, indicating FM homozygotes and heterozygotes exist. The study aim to develop a molecular method for measuring EDN3 copy number and apply it into the breeding. We designed specific primers according to the sequence of target gene EDN3 and reference gene GAPDH, extracted total DNA from blood and amplified the DNA template using quantitative real-time PCR. Six Dongxiang cocks with known EDN3 copy number were chosen and test crossed with 36 ISA layers B line. We also established ten Dongxiang chicken families with homozygous FM. The results showed that the EDN3 amplification curve was standard S-shaped and the amplified fragment was single; so, the designed primer can be used for quantitative analysis. When the sample had a EDN3 copy number value of 2.00 ± 0.12 and 1.50 ± 0.14 , it might be judged as homozygous and heterozygous FM, respectively. The test crosses showed the black skin ratio among Dongxiang homozygous and heterozygous FM offspring was 100% and 47.5%, respectively. The judgement of FM genotype in Dongxiang chicken based upon a test cross and the EDN3 copy number was therefore consistent. Among FM homozygous Dongxiang families, the offspring had all black skin and the average L value of skin color was 56.98 ± 3.52 , significantly different from the control population ($P < 0.05$). This study indicated that the measurement of EDN3 copy number is feasible and reliable. This method will help the breeding of new lines of Dongxiang black-boned chicken.

Keywords: endothelin 3, gene copy number, Dongxiang blue-shell chicken

S2-0056 Incomplete dosage compensation in female not in male of chicken

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Dosage Compensation is an equalization mechanism of organisms to balance gene expression between the males and females. Different species of organisms performed different dosage compensation mechanisms. Birds are able to tolerate a mild male bias for many Z-linked genes, and only equalize expression levels for some genes. To detect the details about this gene-by-gene regulation mode, we have performed the following experiment. Two inbred strains, Cornish and White Leghorns, were chosen to generate reciprocal cross progeny. Three tissues (brain, liver and pectoralis) of 11 chickens (3 males and 3 females in family I, 3 males and 2 females in family II) at the age of 1 day were collected to perform transcriptome sequencing (RNA-seq). At same time, DNA samples of 4 parents from the two families were used for whole genome re-sequencing. With the data of re-sequencing (82.5Gb), we simulated four parental genomes, which could eliminate bias when aligning the RNA-seq data (116.5Gb). To identify which allele did the reads transcribed from, we filtered out 440,631 SNPs between the two parental strains. 38,526 of them located on Z-chromosome. After normalized the reads counts of RNA-seq data, SNPs with sufficient reads covered were chosen to reveal the expression mode of genes. We found that genes on both of two Z-chromosome in male could express, and the number of reads transcribed from two alleles have no significant difference (paired t-test, $P > 0.05$) in three tissues. Expression level of genes on Z-chromosome in male (ZZ) was approximately 1.5-fold of female (ZW), which consistent with previous researches. It confirms that genes on Z-chromosome of female were up-regulated, but inactivity did not happened in male.

Keywords: dosage compensation, RNA-seq, bird, chicken

S2- 0057 Genome- wide association study revealed genomic regions related to white/red earlobe color trait in the Rhode Island Red chickens

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Earlobe color is a naturally and artificially selected in chicken. Redness and whiteness are predominant earlobe color in chicken, and in some breeds, chickens also present with blue, yellow, purple or black earlobes. Naturally, shining earlobe color is negatively selected since it gets more attention of their predators. And also, it is selected by human for keeping the breed characteristics. White/red earlobe color has been found to be related at least three loci and sex-linked. In the present study, we performed genome- wide association (GWA) analysis to explore the candidate genomic regions underlying chicken earlobe color phenotypes in one inbred population of Rhode Island Red chicken in which the earlobe present red, white or red and white. We used hens with red dominant earlobe and hens with white dominant earlobe for case- control analysis by Illumina 600K SNP arrays. The GWA results showed that one 2.38Mb genomic region (50.13 to 52.51Mb) with 282 SNPs being 5% genome- wide significance on chromosome Z were significantly correlated to earlobe color. Sixteen genes were found in the region including PAM, SLCO4C1, ST8SIA4, FAM174A, CHD1, RGMB, RIOK2, LIX1, LNPEP, SHB, RNF38, TRIM14, NANS, CLTA, GNE, CPLX1, and other 7 anonymous genes. Out of the genes, SLCO4C1 belongs to OATP family whose function is related to the pigment transition, making the gene plausible for the trait. Two autosomal regions with 4 SNPs being the suggestive significance also showed to be associated with earlobe color, supporting that the trait was determined by more than two loci.

Keywords: Rhode Island Red chicken, earlobe color, GWAS

S2- 0058 Screening of differentially expressed genes in the growth stage of broiler chickens with Microarray gene expression profiles

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Chinese local chicken breeds Dahan 699 broilers and exotic breeds Recessive White chicken were selected as the object of study, in order to study genes expression changes of Dahan 699 broiler and Recessive white chicken from the overall level at different growth times and the growth and development mechanism, which is of great significance for understanding the molecular mechanisms of chicken muscle growth and development. In this study, gene expression profiles of 2, 6, 10 weeks of age Dahan 699 broiler chicken and White chicken muscle tissues were studied respectively by cDNA microarrays and provide a theoretical basis further study. The Dahan 699 broilers and the White chickens were raised under the same condition. Total RNA samples from 18 chickens (three individual chickens from each group on weeks 2, 6 and 10) were sent to GeneTech Biotech for hybridization to chicken Affymetrix GeneChips (Affymetrix). Briefly, total RNA (500 ng) was purified using a QIA-GEN miRNeasy kit. The identified differentially expressed genes were analyzed for GO and biological pathways using DAVID (<http://david.abcc.ncifcrf.gov>). The main results showed that 17 genes including ADRA1B and MSTN were obtained and they were related with muscle growth and development at 2 week, 12 genes including MUSK and CAPN3 were obtained and they were related with muscle growth and development at 6 week, 27 genes including BMP10 were obtained and they were related with muscle growth and development at 10 week between the Dahan 699 broiler and the White chicken. Our findings demonstrate the chickens muscle growth and development at different growth stage maybe regulated with different genes expression. The differentially expressed genes we identified by microarray analysis will be used in future studies to clarify the molecular regulation mechanisms of chickens growth.

Keywords: chicken, GeneChip, gene expression, muscle growth

S2- 0059 Innovation of breeding scheme and its application to selection of quality chickens

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In China, the quality chicken industry is an important agricultural sector due to the abundant local chicken genetic resources and huge market potential. The quality chicken meat production accounted for 25% of the total output of poultry meat in 2013. Because of the low production efficiency of local chicken breeds, it is very important to set up a breeding scheme to develop crossbred with high production efficiency and excellent meat quality. The objectives of this study were to establish a breeding scheme based on the combined methodology of molecular genetics and quantitative genetics, and to breed for production and meat quality using local chicken breeds. In the past 26 years, the research methods of this study were keeping pace with the development of world genetic breeding technology, we have selected 18 specialized lines using the local chicken and its genes of meat quality, of which five lines have been certified at the provincial level, and the crossbred named Dahan 699 chicken was gained national certificate, which were combined with the characters of appearance and meat quality of local chickens. Pureline selection improved the production efficiency largely. At the age of 10 weeks, the body weight of the crossbred Dahan 699 chicken is 88% higher than that of local breeds, and the feed consumption was decreased by more than 23%. The application of our new crossbred and the supporting technology resulted in significant social, economic and ecological benefits. Dahan chickens were welcomed by the industry and applied in the chicken production in 18 provinces of China. In total the economic benefit of 5.262 billion Yuan was realized in the chicken industry of China. The study won the Second Prize of National Science and Technology Progress Award in 2014.

Keywords: Chinese local chicken breeds, quality chicken, genetics and breeding

S2- 0061 Biological mechanisms discriminating a fast growing high adult body weight and a slow-growing low adult body weight chicken breed

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Intensive selection increased the growth rate and muscularity of broiler chicken, adversely associated with delayed organ development, the sudden death syndrome, and affected metabolic rate. The Wuding chicken breed is a fast growing large chicken breed, and the Daweishan mini chicken breed is a slow growing small chicken breed. Together they form an ideal model system to study the biological mechanisms underlying the broiler chicken selection responses in a natural system. The objective of this study was to study the underlying biological mechanisms differing between the two breeds in muscle and liver tissues, and relate these to the growth and body developmental phenotypes of the two breeds. The muscle tissue showed higher expression of muscle developmental genes in the Wuding breed accompanied by higher expression of immune traits related to the acute inflammatory reaction. The muscle tissue of the Daweishan mini chicken breed showed higher expression of several metabolic mechanisms including endoplasmic reticulum, protein and lipid metabolism, energy metabolism and specific immune traits. The liver tissue showed less differences between the two breeds. Analyses of the genes higher expressed in the Wuding breed showed no specific enriched gene networks and no biological mechanisms were significantly enriched. The Daweishan mini chicken breed showed enriched protein metabolism, ABC receptors, signal transduction, and IL6-related mechanisms. We conclude that fast growth rate large body size related to increased expression of muscle tissue development and activated damaging immune response, which slow growth small body size related to increased general cellular metabolism. The liver of the slow growth rate small body size breed showed a more active metabolism.

Keywords: growth rate, chicken (*Gallus gallus*) breeds, metabolic differences, biological mechanisms

S2-0062 Effect of demotivation and selection on the expression of somatotrophic axis genes and association with body size and growth performance in chickens

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The objective of this study was to investigate effects of domestication and selection on the mRNA expressions of GH/IGF-1 somatotrophic axis genes and association with body size and growth performance in two Chinese native chicken breeds (Daweishan Mini chickens and Wuding chickens), and selection for Avian broilers as control. Total 240 chicks of 1 day of age reared to 12 Weeks. The chickens were sacrificed to measure carcass traits, circulating hormone levels of GH/IGF-1 somatotrophic axis from the blood, and expressions of GH/IGF1 somatotrophic axis genes mRNA from liver and muscle tissues on Week 0, 4, 8 and 12. The growth performance and body size were measured weekly. The results showed Growth performance, body weight and FCE in Mini chickens were significantly lower than Wuding chickens and broilers. Wuding chickens and Mini chickens showed the higher levels of plasma GH, pituitary GH mRNA and lower levels of hepatic GHR mRNA. Selection for fast growing broiler chickens might have been increased skeletal muscle GH binding activity. Moreover, Mini chickens showed significant lower levels of plasma IGF-1, leg muscle and hepatic IGF-1 mRNA compare to broiler and Wuding chickens. It is obviously that domestication effect on growth performance, body weight and

size might via change the profiles of somatotrophic gene expression. IGF-1/IGF1R pathway genes might play an important role in domestication affecting on the growth performance, body weight and size in model domestic chicken breeds. These results demonstrate that domestication and genetic selection for growth performance have altered the expression profiles of somatotrophic axis genes in a breed-, age-, and tissue-specific manner. Consequently, the somatotrophic axis is likely to be a promising target for candidate genetic markers for improving growth performance and body size in chickens.

Keywords: domestication, selection, somatotrophic axis, chickens

S2-0063 Polymorphism identification in HTT genes and its association with production performances in Huangshan Black Chickens

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The aim of this study was to identify genetic polymorphisms of the Huntington (HTT) gene and further investigated its association with production performances in Huangshan Black Chickens. A total of 269 Huangshan Black Chickens of similar age and weight were evaluated in the present study. We extracted DNA from blood samples and analysed polymorphisms of HTT gene as wells as possible association with production performances including age at first egg (AFE), laying rate (LR), egg production (EP), egg weight (EW), average egg weight (AEW). Two polymorphisms (G50831C and G62976A) were detected and located in 42 exon and 54 intron in HTT gene, respectively. The SNP G50831C leads to a synonymous substitution (glutamate acid-to- glutamine). Genotypes and Alleles frequency analysis showed that SNP G62976A genotypic distributions were 0.784 for GG, 0.205 for GA, 0.011 for AA. The genotypic distribution was in Hardy-Weinberg equilibrium (χ^2 -square=0.5021, $P>0.05$). The SNP G50831C genotypic distributions were 0.833 for GG, 0.138 for GC, 0.029 for CC, The genotypic distribution deviated from Hardy-Weinberg equilibrium (χ^2 -square=12.8440, $P<0.01$). The association analysis demonstrated that, in SNP G62976A, chickens with the GG genotype and GA genotype had significant higher LR, EP and EW than individuals with the AA genotype ($P<0.05$). In SNP G50831C, chickens with the GG genotype and GC genotype had significant higher LR, EP and EW than individuals with the CC genotype ($P<0.05$). In conclusion, our study suggest that HTT should be considered as a potential molecular marker for the selection of production performance-related phenotypes in local chicken breeds.

Keywords: HTT gene, polymorphism, local chicken breeds, egg production traits

S2-0064 The characteristic comparison and analysis of Wuding chicken and Avian chicken skeletal muscle satellite cells

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Chicken skeletal muscle satellite cell also called chicken muscle stem cell which located between the basement membrane and the sarcolemma of mature muscle fibers, are closely correlated with chicken skeletal muscle postnatal development particularly plays a key role in chicken muscle hypertrophy and injury induced muscle regeneration. Avian broiler has been genetic selected on high growth velocity and large muscle mass, and the Wuding chicken is a famous local chicken with non-selection breeding and a slow growing in Yunnan Province. The objective of this study was to investigate the effects of selection and breed on the proliferation and differentiation property of satellite cells between the two chicken breeds by immunofluorescence, H&E staining and Real-time PCR. Skeletal muscle satellite cells were isolated from leg muscle, and cultured in proliferation or differentiation medium to compare the characteristics of proliferation and differentiation from the two breeds respectively. It has been showed that the growth curved of satellite cells were in normal growth state with S-shaped, and the Wuding chicken entered into the logarithmic phase and plateau phase were much later one-day than Avian broiler. All the data indicated the skeletal muscle satellite cells of Avian broiler grew and differentiated faster than that of Wuding chicken. We suggest that the selection breeding applied to these breeds regulated the characteristics of the skeletal muscle satellite cells to influence the muscle growth.

Keywords: avian broiler, Wuding chicken, skeletal muscle satellite cells

S2- 0065 Differential regulation of myostatin mRNA expression in muscle tissue of commercial broiler chicken and Yunnan Daweishan mini chicken – Association with growth rate, carcass weight and breast and leg muscle development

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Myostatin is a negative regulator of skeletal muscle growth. Commercial Avian broiler chickens have been selected for high growth and muscularity. And the Daweishan mini chicken is a slow growing small chicken breed. The objective of this research was to investigate the relation between Myostatin mRNA expression level and chicken growth rate, body growth, and muscle growth. Chickens were slaughtered at day 0, 30, 60, 90, 120, and 150 (20 animals per breed per time point), and breast muscle and leg muscle were sampled. The carcass weight, dressing percentage, breast muscle weight, and leg muscle weight were higher at all time points in Avian chicken compared to Daweishan mini chicken. The breast muscle myostatin mRNA expression at 30 days of age was higher in Avian chicken than in Daweishan mini chicken. The Myostatin mRNA expression reached a peak level at day 60 in both muscles in Daweishan mini chicken, after which the expression levels in breast muscle of Daweishan mini chicken remained higher than in Avian chicken breast muscle from day 60 onwards. The myostatin mRNA expression in both muscles correlated positively with the carcass weight, breast muscle weight, and leg muscle weight in mini chicken ageing from 0 to 60 days, and negatively with the carcass and muscle weights of Avian chicken from 90 to 150 days, and body weight of Daweishan mini chicken. We concluded that the myostatin mRNA expression in these chicken breeds seems to be involved in regulation of body growth and muscle development. However, there seems to be two different regulatory mechanisms with a switch around day 60. We suggest that the selection pressures applied to these breeds regulated the myostatin mRNA expression to influence body and muscle growth rate and weight.

Keywords: commercial broiler chicken, Daweishan mini chicken, myostatin (MSTN) mRNA expression, carcass weight, growth rate

S2- 0066 Transcriptome analysis of host response to environmental temperature differences in two genetically distinct chicken inbred lines challenged with Newcastle disease virus infection

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Heat stress causes significant economic losses to the poultry industry and genetics plays an important role in regulating the physiological responses to heat stress. The objective of the current study was to identify genes and signal pathways associated with heat stress through RNA-Seq analysis using two genetically distinct highly inbred chicken lines (Leghorn and Fayoumi). At 14d of age, birds were exposed to 38°C with 50% relative humidity for the first 4 hours, then 35°C till the conclusion of the experiment. Non-treated individuals were kept at 29.4°C throughout the experiment. For the heat stress group, birds were inoculated at 21d with 107 EID₅₀ of Newcastle disease virus (NDV) La Sota strain to investigate the effects of both heat stress and NDV infection. Physiological response in blood measured at four stages: Pre-heat, Acute stress (AS), Chronic stress1 (CS1) and 2 (CS2) at 4h, 7d and 10d post heat treatment, respectively, using the i-STAT device suggested significant difference in response to heat stress between Fayoumi and Leghorn lines. Most i-STAT parameters were significantly changed with temperature differences in Fayoumis but not in Leghorns. Liver samples were harvested on both AS and CS2. Thirty-two liver cDNA libraries (4 libraries per time stage in both Leghorn and Fayoumi) were used to identify differentially expressed genes (DEG) with a FDR < 0.05. Preliminary analysis using DESeq2 identified 90 and 45 DEGs at AS and 142 and 9 DEGs at CS2 in Fayoumi and Leghorn, respectively. IGFBP1 showed opposite expression patterns between the two lines at AS with up-regulation in Fayoumi and down-regulation in Leghorn. Five DEGs were shared by the two lines at CS2, in which 4 out of 5 (MT4, MSMB, AOC1 and GPX1) had opposite regulation in response to combined heat stress and NDV infection. Further data mining will explore more candidate genes associated with thermal tolerance, which can be used for potential genetic improvement of heat stress in poultry.

Keywords: chicken, heat stress, transcriptome analysis, differentially expressed genes, inbred lines

S2-0067 The slaughter traits of quality broilers with different feeding models

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With the rapid development of poultry industry and the change of consumer needs, the feeding model of chicken has converted from the traditional rural cage-free model to the modern intensive caged model, and then returned to the ecological range. Materials and methods: This study we chose a cultivated high quality chicken line as materials to carry out a contrast experiment between stocking model and captivity model and then measured their slaughter traits. Eighty cocks, named S07 line, were allocated into two feeding models at five weeks of age: stocking model vs captivity model. The slaughter traits including weights, dressed weight, semi-eviscerated weights and eviscerated weights, breast muscle and leg muscle, and abdominal fat percentage were measured and then analyzed at the end of 10 weeks of age and 22 weeks of age, respectively. The results showed that the broilers of captivity model had significant higher weights, dressed weight and abdominal fat percentage ($P < 0.01$), semi-eviscerated weights and eviscerated weights ($P < 0.05$) than those of stocking group at both 10 weeks of age and 22 weeks of age. The breast muscle percentage of captivity group was significantly higher, the leg muscle rate was significantly lower, than those of stocking group ($P < 0.05$) at 10 weeks of age, but both indicators had no difference at 22 weeks of age. The results suggested that either stocking model or captivity model, the cocks of S07 line showed an excellent meat character. The meat quality of captivity model was better than that of stocking model, and could significantly promote the storage of abdominal fat; feeding model affected the muscle's growth of different part.

Keywords: quality broiler, feeding model, slaughter traits

S2-0068 Sequencing and genetic feature of BLB2 gene in different chicken breeds

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Major histocompatibility complex (MHC) is closely linked by a group of highly polymorphic loci, which induces the transplant rejection, presents antigen peptides to T cells for recognition and triggers immune responses. In domestic poultry, depending on the type of MHC molecule encoding protein, the MHC molecules are divided into three categories: B-F, B-L, B-G. The B-L gene is one of the most abundant areas of MHC gene polymorphism in MHC system. The purpose of this study was to find out the differences of BLB2 gene in different chicken and identify potential functional sites. In this study, BLB2 gene were sequenced by using target sequence capture assay in different chicken breeds, then SNPs, Indels and evolutionary aspects for these different chicken breeds were analyzed. Furthermore, according to the clustering analysis and the obtained haplotypes of some chicken breeds, we explored the relation between polymorphism of BLB2 gene and disease-resistance. The results showed that the polymorphism of BLB2 gene mainly occur in the exon 2. Furthermore, the results of phylogenetic analysis showed the polymorphism within this region can contribute to the determine of MHC haplotypes. And there is very close relationship between MHC haplotypes and the disease-resistance. The screened SNPs and Indels can be used as the candidate resistant marker. Collectively, these results provide molecular data for the study on immune function and genetic evolution of chicken BLB2 gene and provide reference for the development of resistance marker-assisted selection (MAS) in avian MHC region.

Keywords: MHC, BLB2, indigenous Chinese chicken, genetic polymorphism

S2-0069 Analysis of genetic diversity and population structure of 4 Anhui indigenous chicken breeds using microsatellite markers

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Anhui province has a wide variety of indigenous chicken resources. This study aims to more reliably assess genetic diversity and estimate the genetic structure of Anhui indigenous chicken resources. A total of 370 individuals originating from 4 Anhui indigenous chicken breeds (Huainan partridge chicken, HN; Huoqiu chicken, HQ; Jinzhai black chicken, JZ; Huangshan black chicken, HS) were analyzed in this study. A total of 21 microsatellite markers were selected to screen all of the samples. The analysis of genetic diversity and population structure were estimated by Microsatellite-Toolkit for Excel, Dispan and Structure. A total of 208 alleles were observed in the 4 chicken breeds. The number of alleles per locus ranged from 4 (MCW0020 and MCW0222) to 28 (LEI0234). The polymorphism information content (PIC) of alleles per locus ranged from 0.142 (MCW0081) to 0.924 (LEI0234). The lowest estimate of expected heterozygosity (0.650) was obtained for HS, while the highest one (0.668) was found in HQ. The lowest observed heterozygosity was 0.587 (Huangshan black chicken), while the highest one was (0.667) (Huoqiu chicken). The Nei's genetic distance values ranged from 0.0212 (between HN and HQ) to 0.2187 (between HN and HS). A phylogenetic consensus tree was constructed using the genetic distance data and UPGMA methodology based on genotyping results. The tree indicated that the 4 Anhui indigenous chicken breeds were divided into two categories. HN and HQ were clustered into one group, while the HS and JZ were clustered into the other group. The results of the clustering analysis using STRUCTURE indicated that: the population structures of HN and HQ were similar, the population structures of HS and JZ were different. The results of our study demonstrated high genetic diversity was observed in the 4 Anhui indigenous chicken breeds, and it will play an important role in conservation, supervision, and utilization of the Anhui chicken resources.

Keywords: Anhui chicken, microsatellite markers, genetic diversity, population structure

S2-0070 Study of the feather maturity associated with VEGF and VEGFR 2 expression in Qingyuan partridge chicken

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Vascular endothelial growth factors (VEGF) and its receptors (eg. VEGFR 2) induced angiogenesis in the development of hair follicle. In order to understand the molecular mechanism of chicken molting, the skin histological structure of feather follicle and the expression of VEGF and VEGFR-2 gene were detected, which were useful for chicken breeding and processing. Qingyuan partridge chickens at 120 days (n=12) were used for follicle microstructure investigation by H.E staining. The expressions of VEGF and VEGFR-2 gene were detected and analyzed by RT-PCR and SPSS16.0. The bulky pinna rachis with blood vessels and feather pulp liquid, the thicker feather wall, the vascular density with calamus deep into skin, and relatively mature dermal papilla were observed in the growth feather follicle, which named the immaturity feather. The maturity feather showed the contraction refinement pinna rachis without feather pulp liquid, calamus vasoconstriction with loosely packed in skin. In the immaturity feather, the average follicle diameter was 116.94 μm bigger, and the average follicle depth was 178.05 μm higher than that in the maturity feather. The average density of feather follicle in mature feather was higher 2.34 unit/cm² than that in the immaturity feather. The skin expression of VEGF and VEGFR-2 in the immaturity feather was 2.49-fold and 1.62-fold higher than that in the maturity feather ($P < 0.05$), respectively. The maturity feather is associated with feather molting and different development histological structure. The higher expression pattern of VEGF and VEGFR-2 gene in skin was similar in the immaturity feather versus the maturity feather, suggesting two genes may be positive related to angiogenesis, and could be candidate genes for feather development.

Keywords: chicken, feather maturity, expression, VEGF, VEGFR-2

S2-0071 High-throughput sequencing reveals circulating miRNAs as potential biomarkers for measuring puberty onset in chicken

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There are still no highly sensitive and unique biomarkers for measurement of puberty onset. Circulating miRNAs have been shown to be promising biomarkers for diagnosis of various diseases. To identify circulating miRNAs that could be served as biomarkers for measuring chicken puberty onset, the Solexa deep sequencing was performed to analyze the miRNA expression profiles in serum and plasma of hens from two different pubertal stages, before puberty onset (BO) and after puberty onset (AO). 197 conserved and 19 novel miRNAs (reads > 10) were identified as serum/plasma-expressed miRNAs in the chicken. The common miRNA amounts and their expression changes from BO to AO between serum and plasma were very similar, indicating the different treatments to generate serum and plasma had quite small influence on the miRNAs. 130 conserved serum-miRNAs were showed to be differentially expressed (reads > 10, $P < 0.05$) from BO to AO, with 68 up-regulated and 62 down-regulated. 4829 putative genes were predicted as the targets of the 40 most differentially expressed miRNAs ($|\log_2(\text{fold-change})| > 1.0$, $P < 0.01$). Functional analysis revealed several pathways that were associated with puberty onset. Further qRT-PCR test found that a seven-miRNA panel, including miR-29c, miR-375, miR-215, miR-217, miR-19b, miR-133a and let-7a, had great potentials to serve as novel biomarkers for measuring puberty onset in chicken. Due to highly conserved nature of miRNAs, the findings could provide cues for measurement of puberty onset in other animals as well as humans.

Keywords: chicken, puberty onset measurement, circulating miRNA

S2-0072 Genetic variation of HIF2a gene in Tibetan chicken and seven low-land chicken breeds in China

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The problem of hypoxia adaptation in high altitudes is a hot spot in the field of sciences. As one of the best chicken breeds with adaptability to highland environment, the Tibetan chicken, is genetically different from lowland chicken. To understand the mechanism of hypoxia adaptability in high altitude, we focused on the HIF2a, also called EPAS1, which is the key regulatory factor of hypoxia response. In this study, to detect the polymorphisms of HIF2a CDS area in 296 individuals from 8 Tibetan chicken populations and 7 Low-land chicken breeds, 15 pairs of primers were designed and amplify using DNA pooling and DNA sequencing methods. Seven single nucleotide polymorphisms (SNPs) were detected and we found a missense mutation (rs316126786 A/G) resulting an amino acid mutation. Besides, the rs14330062 C/T was unique in Tibetan chicken. After compared the genotype between Tibetan chicken and Lowland chicken, three of them showed a big difference. Interestingly, birds with AA genotype for the SNP4 exhibited significantly increased in Tibetan group, while AG and GG showed opposite tendency. We speculated the possibility of this mutation might be harmful to energy metabolism in Tibetan chicken but lowland chicken can bear it. Therefore, we considered that the polymorphism (SNP4) in the HIF2a gene may affect the specific functions of EPAS1 protein relating to high-altitude adaptation of Tibetan chicken.

Keywords: HIF2a, high- altitude adaptation, SNPs, genotype

S2-0073 Genetic analysis of Huoyan trait in the Huoyan goose

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Owing to its good laying performance, Huoyan goose is a valuable native poultry genetic resource in China. As the breed characteristics of Huoyan goose, it is not known about inheritance patterns of Huoyan trait yet. The purpose of this study was, by establish the goose-F2 resource group with Huoyan trait record, to test our hypothesis that the allele that determines Huoyan trait demonstrates recessive inheritance, furthermore, to understand the genetic mechanism of Huoyan trait. F1 generation of Huoyan goose pure line by random mating. By F2-design of out-breed populations, the resource population with Huoyan trait record established. Huoyan geese were crossed reciprocally with Sichuan White goose, and inter se mating in F1 was carried out to generate the F2 population. Huoyan appearance and segregation ratio in F1 and F2 were observed. Huoyan phenotypes in F1 generation of Huoyan goose pure lines by random mating was 86% , in which percentage of Huoyan trait in male and female geese were 83%, 90%, respectively. All the males and females in reciprocal cross F1 populations were normal eyelid. In F1 populations of Huoyan goose gander crossed with Sichuan White goose female, all the males were normal eyelid, the females with Huoyan and with normal eyelid trait were 83%, 17%, respectively. By the χ^2 test, the proportion of differentiation of the F1 resource group was accorded with the law of independent segregation of classic Mendelian inheritance. In F2 populations of Huoyan goose crossed reciprocally with Sichuan White goose, real ratio of Huoyan vs normal eyelid phenotype in males and females were 5:8, 2:3, 0:1 and 5:7, respectively. By the χ^2 test, the proportion of differentiation of the F2 resource group was accorded with the laws of classic Mendelian inheritance. Huoyan trait exhibit recessive heredity to normal eyelid trait, and the allele that determines Huoyan trait demonstrates sex-linked inheritance.

Keywords: Huoyan trait, recessive inheritance, sex-linked inheritance, goose-F2 resource population, Huoyan goose

S2- 0075 Genome- wide association study identifies loci for bone traits in chickens

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Osteoporosis in chicken is a common disease characterized by an increased risk to fracture due to reduced bone mineral density (BMD) and deterioration of the structural bone tissue. Skeletal problems, associated with osteoporosis, are gaining attention due to animal welfare and economic losses in the chicken egg industry. Currently, nutritional supplements, especially with regard to calcium and vitamin D intake, as well as furnished cages to increase physical exercise are used to prevent osteoporosis. But, genetic improvement has been proposed as an promising method to resolve the skeletal issues. Recently, we performed a genome- wide association study (GWAS) in a crossbred population. Our objective was to identify genomic markers and loci associated with bone traits in chicken. Using Bonferro-ni-corrected p-values ($p < 8.43 \times 10^{-7}$) as the standard for genome- wide significance, we found significant associations with osteoporosis-related traits for several SNPs, consisting of six intergenic SNPs, three intronic SNPs (in INTS6, POSTN, and FGFBP1) and one missense coding SNP (in SERPINE3). Three of these genes, RANKL, ADAMTS, and SOST, were known to be associated with osteoporosis in humans, which made them extremely good candidate genes for osteoporosis in chickens. A novel locus close to GSG1L, associated with femur BMD, was uncovered in this study. Genomic partitioning analysis supported the common variants contributing to variations of bone-related traits. In addition, we found a genomic region on GGA 1 that had pleiotropic effects on all bone traits. Using a linear mixed model approach, we identified several strong candidate genes and genomic regions associated with bone traits measured in end-of-lay cage layers, which accounted for 4.7 %~7.2 % of phenotypic variance. These SNPs could provide relevant information to help elucidate which genes affect osteoporosis in chickens.

Keywords: GWAS, osteoporosis, BMD, bone breaking strength, layer

S2- 0076 Quantitative genetic analysis of a breeding process of Pekin duck in eleven years

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Body weight (BW) and breast muscle weight (BMW) are the main selected traits in duck breeding programs. The aim of this study was to analyze and estimate genetic parameters and breeding values of BW, BMW, and breast muscle percentage (BMP), respectively. The three traits of 15998 ducks at 6 wk age were determined in eleven years (2005-2015), which were from Chinese Academy of Agricultural Science. Genetic parameters and estimated breeding value (EBV) of these three traits were estimated using a multivariate model with MTDFREML and PEST respectively. The model included fixed effects (year and sex), additive genetic effects, and residual effects. A high heritability (0.45) was observed in BW, while a medium heritability (0.23 and 0.12) was found in BMW and BMP. BMW showed high genetic and environmental correlation with BW (0.87 and 0.69) and BMP (0.64 and 0.84), whereas the genetic and environmental correlation between BW and BMP was very low (0.15 and 0.13). Substantial progress was observed in genetic modification of all these three traits during the eleven years. From 2005 to 2015, the annual average EBV increased by about 345 in BW and 44 in BMW. The correlation coefficients of BW and BMW between selection intensity and annual increased EBV were 0.83 and 0.63, respectively. These genetic parameters indicated that the selection for BMW could also increase both BW and BMP. Given the high correlation between selection intensity and annual increased EBV, the breeding effects could be directly influenced by the selection intensity. This research combined the genetic parameter and EBV with actual breeding progress, and it may provide some guidance for actual breeding process.

Keywords: genetic parameter, estimated breeding value, selection intensity

S2- 0077 Expression of brain- derived neurotrophic factor in pre- hierarchical follicles of geese

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Brain- derived neurotrophic factor (BDNF) is one of the neurotrophic factor family members. It has been demonstrated that BDNF plays a role in regulate the development of follicles. According to the follicular diameter, poultry pre-hierarchical follicles were graded as large white follicles (6~8mm), middle white follicles (4~6 mm), small white follicles (2~4 mm) and primary follicles(<2 mm). We selected Zi geese pre-hierarchical follicles as the study objects to explore the expression of BDNF in pre-hierarchical follicles of geese. According to geese reproduction cycle, 12 Zi geese 35-37 weeks of age were used in this study. The pre- hierarchical follicles were washed in phosphate buffered saline and placed in liquid nitrogen. Total RNA was extracted from 4 stages of pre-hierarchical follicles of Zi geese and synthesized cDNA with reverse transcription. Then we used RT- PCR technique to quantify the expression of BDNF in 4 stages of pre- hierarchical follicles. Results as follows: BDNF exists in 4 stages of pre-hierarchical follicles of geese, with the highest expression in primary follicles. The expression of BDNF in primary follicles is significantly higher than that of other models ($P<0.05$). The rest follicles is not significant ($P>0.05$). We conjectured that BDNF exists at the beginning of follicular development and involved in regulate the development of follicles. But this study did not detect the expression of BDNF receptor in pre-hierarchical follicles. Whether BDNF and its receptor regulate follicles by paracrine has to be studied further.

Keywords: BDNF, RT-PCR, pre-hierarchical follicles, geese

S2-0078 Egg production performance of Rhode Island Red, Bashang Long-tail chicken and their reciprocal crosses progenies responses to cold environment

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Cold environment affects homothermal animals seriously, which can significantly reduce chicken egg production performance. Bashang long-tail chicken (BS), originating from Bashang in Hebei province, is deemed as cold tolerance due to the climate of Bashang region. Rhode island red chicken (RD) and the reciprocal crosses progenies of BS and RD were also used in this study. All 4 groups of chicken, from 20 weeks of age, were reared in housing stair-step cages and half-open shed during the winter from September 2015 to January 2016. During whole study period, the house temperature decreased slightly from about 25°C to 10°C, but the ambient temperature of half- open shed decreased sharply from about 25°C to -15°C with natural temperature fluctuations. In housing cages, hen-day egg performance rate (HDEP) to 40 weeks of age of RD (87.1%) was higher than that of BS (62.2%, $p<0.01$). While in half- open shed, HDEP was nearly equal between RD and BS when the ambient temperature was relative warm from September to November 2015. But the ambient temperature was below 0°C from December 2015, and HDEP from 30 to 40 weeks of age of BS (60.4%) was higher than that of RD (44.6%, $p<0.05$) over this cold period. The reciprocal crosses progenies were greatly improved in egg production performance and cold tolerance. In further study, we have fund cloacal temperature of BS was lower than that of RD, and blood thyroid hormones T3 and T4 level of BS was higher than that of RD ($p<0.05$). This primary study has shed some light on the understanding of cold tolerance.

Keywords: Bashang long-tail chicken, Rhode Island red chicken, cold tolerance, egg production performance

S2- 0079 Analyzing on breeding progress of Dahan slow feathering lines S06M

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Chicken fast-feathering and slow-feathering belong to auto-sexing traits, it is often used for chicks distinguish gender. Its advantages are simple, fast, accurate, labor saving, saving the cost of human resources, and can avoid double anal to identify the stress reaction of chicks and the spread of disease, chicks damage, etc. But there are few auto-sexing crossbreed which is genetically stable and has good production performance in China. Therefore, we started to the breed selection and conservation for slow-feathering lines. After five generations of breeding, genetic tests showed that gene pure coverage reached 100% for slow-feathering group, 98.5% homozygous phenotypes, and the rooster average weight is 2340.85 ± 328.60 g, the hen average weight is 2048.21 ± 195.46 at 10 weeks, 68 weeks egg production is 168.7. In addition, the lines pockmarked proportion was 93.1%, and black shank proportion was 95.5%. Our results laid a solid foundation for cultivate speeding of the slow-feathering lines.

Keywords: Dahan, slow-feathering, production performance

S2- 0080 Comparative analysis of bone stability of four layer lines kept in two husbandry systems

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Selection for high egg production is seen as one of the main causes of the occurrence of osteoporosis in laying hens. The aim of the study was to evaluate the effect of differences in performance level and housing system on bone stability of four lines of laying hens. Within two consecutive test trials from September 2012 to April 2015, two brown (BLA, L68) and two white egg layer lines (WLA, R11), differing in performance level and phylogenetic origin have been studied. After a 16-week rearing period in floor keeping, 192 pullets per generation were transferred to a single cage system. Another 168 and 120 hens in the first and second trial, respectively, were moved to six compartments of a floor keeping system. At the end of the 74th week of age, ten hens per line and husbandry system were sacrificed and assessed for several bone traits, including tibia and humerus (2nd trial) length, weight and breaking strength, bone mineral density (BMD) and content (BMC), medial tibia thickness and the area of bone cortex and marrow, respectively. The analysis of variance showed a highly significant effect of the housing system and layer line on all of the examined traits. Hens housed in floor pens had significantly ($P < 0.001$) higher bone breaking strengths, BMC, BMD as well as absolute and relative cortex areas compared to layers kept in single cages. Although the brown egg layers had significantly longer and heavier tibiae than the white egg layers, the absolute and relative cortex area was higher for R11 and WLA. Tibia breaking strength was significantly lower in hens of the high performing layer lines (WLA, BLA) than in the low performing lines R11 and L68 irrespective of the housing system tested. In conclusion, bone stability primarily depends on the level of motion activity of hens in different housing systems. Moreover, the laying performance of divergently selected layer lines is a major factor affecting tibia breaking strength.

Keywords: laying hen, bone breaking strength, laying performance, phylogeny, housing system

S2–0081 Selection for Feed Efficiency in Ducks

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The global production of duck meat was roughly 4.3 million tons in 2012. It represents a valuable source for human consumption especially in Asian countries. To improve the efficiency of this business, breeding companies have been selecting for superior performance in all traits of economic importance. Selection is focused on daily gain, meatiness and feed efficiency of broiler ducks, without neglecting the number of ducklings per female housed. Improving the feed or nutrient conversion is a key tool to increase the production efficiency. Sophisticated technology to test the individual feed intake and the feeding behaviour of Peking and Muscovy Ducks, developed and used over the last decade was the pre-disposition and driving factor to gain success and more knowledge in feed selection.

Keywords: ducks, feed efficiency, behaviour

S2– 0082 Gene expression profiling of the immunized chicken tissues after heat stress by RNA-seq

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We investigated the presence of differentially expressed (DE) genes in chicken by RNA-seq before and after subjecting the immunized chicken to heat stress. The function and pathway categories of the DE genes were then characterized using bioinformatics techniques. Comparison of the results of pre-and post-heat stress gene profiling revealed 35 significantly DE genes expressed in the spleen tissues (fold change ≥ 2 , $Q \leq 5\%$), of which 19 genes had gene annotations; 30 had modified biological processes such as platelet activation, cholesterol metabolic process, protein polymerization and cellular protein complex assembly; and 7 had distinct biological pathways such as chemokine signaling pathway and vitamin digestion and absorption. In addition, 4 significantly DE genes were detected in the bursa of fabricius tissues (fold change ≥ 2 , $Q \leq 5\%$), of which 4 genes had gene annotations; 2 had altered biological pathways such as protein digestion and absorption and synaptic vesicle cycle. All together, we found 39 significantly DE genes after subjecting the chicken to heat stress; different tissues showed different and specific expression patterns. Our results provide an evidence for the molecular mechanism of heat stress in chicken as well as provide a system to screen heat tolerance-related genes.

Keywords: RNA-seq, heat stress, immunized chicken, differentially expressed

S2- 0083 Genome- wide association studies for intestine length in F2 population of chickens

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Intestine length is an important physiological index. It has effect on nutrient intake and thus plays roles in growth and egg laying in chickens. Although there is some literature about intestine length, little information is available for understanding genetic architecture of it. The current study was conducted to investigate the genetic architecture of intestine length. A total of 1512 F2 hens from a White Leghorn and Dongxiang reciprocal cross were phenotyped for length of intestine including duodenum, jejunum, ileum, cecum and rectum, and genotyped using the chicken 600 K single nucleotide polymorphism (SNP) genotyping array. SNP-based heritability estimation was performed by SAS algorithm and univariate genome- wide association studies (GWAS) were performed by GEMMA, a genome-wide efficient mixed model association algorithm. The length of intestinal parts jejunum and ileum exhibited high SNP- based heritability estimation (0.43, 0.49), while the heritability estimation is moderate for duodenum and cecum length (0.36, 0.39) and low for rectum length (0.19). Five independent univariate genome- wide screens for these five intestine length suggested 202, 298, 119 and 54 SNPs significantly associated with previous four parts of intestine length, respectively, but no SNPs with rectum length. The significant genomic regions for duodenum length ranged from 166.01-172.29Mb on GGA1 and 66.51- 67.43Mb on GGA2; for jejunum length ranged from 166.61-173.26Mb on GGA1 and 73.16- 74.36Mb on GGA4; for ileum length ranged from 166.28-171.57Mb on GGA1, 73.16- 74.36Mb on GGA4, and 1.37Mb on GGA17; and for cecum length ranged from 166.66-171.06Mb on GGA1, indicating ~170Mb on GGA1 is an important region for intestine length. Intestine length exhibited good SNP-based heritability estimation except rectum length and GWAS indicated the important genomic region was on GGA1.

Keywords: chicken, intestine length, SNP, GWAS, genetic architecture

S2-0084 Discovery of piRNAs pathway associated with early-stage spermatogenesis in chicken

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Piwi-interacting RNAs (piRNAs) play a key role in spermatogenesis. Here, we describe the piRNA profiling of primordial germ cells (PGCs), spermatogonial stem cells (SSCs), and the spermatogonium (Sp) during early- stage spermatogenesis in chicken. We obtained 31,361,989 reads from PGCs, 31,757,666 reads from SSCs, and 46,448,327 reads from Sp cells. The length distribution of piRNAs in the three samples showed peaks at 33 nt. The resulting genes were subsequently annotated against the Gene Ontology (GO) database. Five genes (RPL7A, HSPA8, Pum1, CPXM2, and PRKCA) were found to be involved in cellular processes. Interactive pathway analysis (IPA) further revealed three important pathways in early-stage spermatogenesis including the FGF, Wnt, and EGF receptor signaling pathways. The gene Pum1 was found to promote germline stem cell proliferation, but it also plays a role in spermatogenesis. In conclusion, we revealed characteristics of piRNAs during early spermatogonial development in chicken and provided the basis for future research.

Keywords: piRNAs, chicken, germline stem cell

S2- 0085 Identify pathogenesis and categorization of chicken asthenospermia from histology

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Asthenospermia is a common genetic disease in poultry, but little information could found describing its essential characteristics so far. Accordingly, this study aims to explore chicken asthenospermia histology categorize and pathogenesis in field model and artificial model of asthenospermia. With symptoms deepening, there were significant differences between field asthenospermia rooster and normal rooster in semen volume, sperm quality and the rate of sperm deformation ($P \leq 0.05$). Furthermore, after busulfan treated, there were significant difference between artificial asthenospermia roosters and control birds in semen volume, sperm quality and deformation sperm rate ($P \leq 0.05$). In addition, epithelial cell layer, spermatid layer and spermatocytes layer of seminiferous tubule in asthenospermia roosters was strip layer by layer in field model and artificial model. Semen smears also showed that there was significant difference in sperm density among the field model, artificial model and normal ($P \leq 0.05$). In conclusion, we provide a successful way to construct chicken artificial asthenospermia model, and the results demonstrated that the damage of seminiferous tubule structure make testicular tissue spermatogenesis loss, which caused asthenospermia.

Keywords: chicken, asthenospermia, artificial model, histology, pathogenesis

S2- 0086 The candidate gene selection of blue-shelled character in the Dongxiang chicken

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In recent years, blue-shelled eggs are more favorite for consumers in China. Eggshell color is belong to limited character, only in the individual open postpartum to observe, so conventional breeding methods to select purification blue-shelled group is difficulty and slowly. It has found that the blue eggshell phenotype in chickens is caused by a retrovirus insertion in the 5' flanking region of SLCO1B3 coding a membrane transporter OATP1B3 which is responsible for transporting amphipathic organic compounds including bile salt. In this study, based on the previous studies, we examined 323 individuals of the Dongxiang chicken breeder group, of which ♀ 286 and ♂ 37. Blue eggshell color exhibits an autosomal dominant inheritance. The results indicated that the dominant homozygous individuals were 174, 126 heterozygous and 23 recessive homozygote. It's according to the Mendelian law. The hen of homozygous dominant and heterozygotes all laid the blue-shelled egg, so we separate these two genotypes of hen to feeding, and eggs laid by homozygotes are a darker blue than those from heterozygotes. Conventional breeding methods to select purification blue-shelled group is not only a long breeding cycle, also need to invest a lot of manpower and financial resources, using the method of molecular markers can rapidly improve SLCO1B3 gene homozygous genotype frequency, speed up the blue-shelled egg traits purification speed, and the accuracy is high.

Keywords: blue-shelled eggs, OATP1B3 gene, candidate gene selection, breeding, Dongxiang chicken

S2- 0087 Genome- wide association study for beak deformity trait in chickens

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Beak deformity (BD, normally crossed beaks) was found in some indigenous chickens in China. Birds with deformed beaks have reduced feed intake, impeded growth rate, and poor production performance. BD represents an economic as well as an animal welfare problem in poultry industry. The genetic basis of this malformation remains incompletely understood. A case-control GWAS for this trait was performed using a program ROADTRIPS (Version 1.2) in the present study. A number of 48 BD (case) and 48 normal (control) Beijing-You chickens were genotyped using Affymetrix 600K SNP chip. Ninety-five individuals and 429,539 SNPs were available in the association analysis after quality control (QC criteria: call rate > 90%; sample missing rate < 10%; SNP missing rate < 10%; MAF > 0.05). P-Value was corrected by a strict Bonferroni adjustment based on LD pruning. The results revealed that one SNP located on GGA 3 was significantly associated with this trait (5% genome-wide significance). Seven suggestively SNPs located on GGA 1, 3, 5, 6, 10 and 23, respectively, were also identified. These SNPs were located within a gene or located upstream or downstream of several nearest genes, respectively. Combined with the previous digital gene expression (DGE) study, LOC421892 and TDRD3 genes were identified as candidate genes. It was reported that the origin and evolution of beak in birds were strongly associated with neural crest cell and that RET gene played a crucial role in neural crest development. Similarly, STMN1 gene was related to chick retina. The overexpression of this gene conferred an independent prognostic indicator in nasopharyngeal carcinoma. Taken together, from these significant findings, four genes, LOC421892, TDRD3, RET and STMN1, were firstly identified as candidate genes underlying this trait in chickens which require further function verification studies.

Keywords: Beijing-You chickens, beak deformity, GWAS

S2- 0088 Genome-wide association studies for intestine weight of chickens

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Feed costs represent the main costs of raising chickens. The main way to decrease these costs is to improve feed efficiency by modification of diet formulation, but one other possibility would be to use genetic selection. This study was conducted to detect genomic regions that are related to intestine weight with univariate genome- wide association studies (GWAS) by 600 K Affymetrix chicken SNP arrays, using an F2 resource population (1512 hens) phenotyped for intestine weight from a White Leghorn and Dongxiang reciprocal cross. Univariate GWAS were performed with GEMMA, a genome-wide efficient mixed model association algorithm. The statistical significance threshold for association was inferred by the simpleM method. The SNP-based heritability estimates of intestine weight of duodenum, jejunum, ileum and rectum were 0.31534, 0.278382, 0.255602 and 0.337418, respectively, which showed that the intestine weight are under genetic control nearly at the moderate level. Independent univariate genome-wide screens for duodenum weight, jejunum weight, ileum weight and rectum weight detected 185, 201, 36 and 3 significant SNPs, respectively. The significant genomic regions for duodenum weight were ranged from 166.7-173.2Mb on GGA1 and 49.86Mb on GGA4; for jejunum weight and ileum weight were ranged from 166.7-173.2Mb and 167.6-170.2Mb, respectively; for rectum weight were ranged from 79.25Mb on GGA3, 3.31Mb on GGA15 and 1.37 Mb on GGA17. The results showed that intestine weight exhibited moderate SNP- based heritability estimates and identified several regions of the chicken genome involved in the control of intestine weight. It will greatly advance our understanding of the genetic basis underlying intestine and further studies are necessary to identify the underlying genes to validate these in commercial populations and breeding environments.

Keywords: chicken, intestine weight, GWAS, SNP, genetic architecture

S2- 0089 Myofiber Characteristics and transcriptome profiling of skeletal muscle between chicken breeds differing in meat quality

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Background In the modern chicken industry, fast-growing broilers have undergone strong artificial selection for muscle growth, which has led to remarkable phenotypic variations compared with slow-growing chickens. However, the molecular mechanism underlying these phenotypes differences remains unknown. **Methods** In this study, a systematic identification of candidate genes and new pathways related to myofiber development and composition in chicken soleus muscle has been made using gene expression profiles of two distinct breeds: Qingyuan partridge chickens (QY), a slow-growing Chinese breed possessing high meat quality and Cobb 500 broilers (CB), a commercial fast-growing broiler line. Agilent cDNA microarray analyses were conducted to determine gene expression profiles of soleus muscle sampled at sexual maturity age of QY (112 d) and CB (42 d). **Results and Conclusions** 1318 genes with at least 2-fold differences were identified ($P < 0.05$, $FDR < 0.05$, $FC \geq 2$) in soleus muscles of QY and CB chickens. Differentially expressed genes (DEGs) related to muscle development, energy metabolism or lipid metabolism processes were examined further in each breed based on Gene Ontology (GO) analysis, and 11 genes involved in these processes were selected for further validation studies by real-time RT-PCR. In addition, based on KEGG pathway analysis of DEGs in both QY and CB chickens, it was found that in addition to pathways affecting myogenic fibre-type development and differentiation, energy metabolism were also enriched and might form a network with pathways related to muscle metabolism to influence the development of myofibers. This study is the first stage in the understanding of molecular mechanisms underlying variations in poultry meat quality. Large scale analyses are still required to validate the role of the genes identified and ultimately to find molecular markers that can be used for selection or to optimize rearing practices.

Keywords: microarray, gene expression, skeletal muscle, chicken

S2- 0090 Genome sequencing strategy to determin the polydactyly candidate gene in the Bejing Fatty chicken

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Polydactyly (Po) is a common limb malformation in vertebrate characterized by supernumerary digits. To locate the Po gene, a pure five-digit line of Beijing Fatty chicken (BF) was built through 3 years of high strength artificial breeding. A RAD based sequencing was applied to the F2 resource population derived from backcross of male pure five-digit BF with female Shiqi Miscellaneous (SM). A total of 112 chicken from three generation were sampled, consisted of 48 cases (Po) and 64 controls, belong to 11 families. With HindIII digestion, the constructed RAD tags libraries were sequenced by Hiseq 2000. A total of 767,810 high quality SNPs were identified, used to GWAS for polydactyly performed with GCTA using the mixed linear model. All the significant SNP markers associated with Po were focused on chromosome 2 short arm, range from 7 Mb to 9Mb. Given the coverage limit of RAD, whole genome sequencing, with theoretical depth of 30-fold, was applied to 3 Po samples and 3 wild type samples assumed to be homozygous to scan all of the mutation. The G/T mutation of rs80659072, which was reported to be highly associated with Po in silkies chicken, was also identified in our resequencing and validated in random samples. Additionally, to find the specific genomic region most likely to have experienced high strength artificial selection, an outlier-based approach was employed. The biggest F_{st} values exceeded the 99.9% level of the empirical distribution gathered in region ranged from 8.3 Mb to 8.7 Mb, where candidate Po gene LMBR1 located in. In conclusion, the results of GWAS with RAD based markers and resequencing highly suggested LMBR1 to be the cause gene of polydactyly. Our study here-in lay a preliminary foundation for further elucidation of the mechanism of polydactyly.

Keywords: polydactyly, Beijing Fatty chicken, RAD, GWAS, resequencing

S2- 0091 Investigation the effect of thermal manipulation on early embryonic development and during PGC cultivation in domestic fowl

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High environmental temperature can be a stress factor for poultry, thus it can cause reduced productivity traits. The animals can be protected from such negative effects with increased adaptation capabilities through the usage of special keeping technologies. Therefore during this study our aim was to investigate the impact of heat treatment and heat shock on chicken embryos and on chicken PGCs by using in vitro culture system. We performed our experiments on the Transylvanian Naked Neck breed. Three different types of experimental groups were used: heat treated and heat stressed (EK), non-heat treated, but heat stressed (EKNK) and non-treated, non- stressed (EGTK). The main embryological, spermatological and productivity parameters were measured regularly. PGC cultures were derived from blood which was isolated from 2.5- day (HH16) embryos, then samples were collected after 23, 30 and 50 days of culturing. DNA and RNA were isolated at each time points and samples were prepared for immunostaining. In the heat-treated group significantly less abnormal embryonic development was observed when the parents were heat stressed. The heat treatment also caused significant growth in the egg production. Difference was not found in the percentage of abnormally developed embryos. The heat treatment did not have any effect on the spermatological parameters. In the treated group almost twice as many eggs (46.8 %) were experienced, which were appropriate for the PGC culturing, than in the non-treated group (29.8 %). In EK and EKNK populations derivation rate was approximately the same (72.3 % and 78.6 %), however in the EGTK group it was 60%. We could established 39 PGC cultures from the used 56 embryos. 29 cell lines were frozen. The expression of the pluripotency markers and the HSP70 heat shock protein were detectable in the cell cultures. The expression of the HSP70 was considerably higher in the gonads of EK and EKNK embryos than in the EGTK group.

Keywords: thermal manipulation, heat shock, chicken primordial germ cells, cell culture, transylvanian naked neck

S2- 0092 Performance effect of LYZC alleles in Rhode Island Red chicken

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C-type lysozyme gene (LYZC) is located within a short locus of 24 kb of chromatin in chicken that contains all the elements required for position-independent and tissue-specific expression of the gene and displays enhanced general DNaseI sensitivity between two matrix attachment regions. This research analyzed effects of two LYZC alleles for improving embryonic vitality, chicken viability, egg quality and production. The Rhode Red Chicken was feed in Shanghai Poultry Breeding Company Ltd, two alleles were found in a line of Xinyang Black Feather Layer Package. With 3 hybrid roosters and 30 hybrid hens from a line of Rhode Island Red, that were mated and the eggs were hatched for several shifts, and 116 day-old females were wing-banded for brooding and performance test. DNA was extracted from the blood of day-old chicken toes, LYZC allele, L1 and L2, was detected with PCR. Embryonic vitality, chicken viability, egg quality and production were recorded and counted. Activity of lysozyme from egg white and tears of the hens was detected. Result: Statistical analysis showed L1L1 and L1L2 have better performance than L2L2. The embryos of L2L2 were easier dead in the hatching period, statistical significance especially when longer storage of hatch egg. Layer viability of layers from day-old to 18 weeks age were 97%, 98% and 79% corresponding to gene types of L1L1, L1L2 and L2L2. The first egg weight of L2L2 was 0.6 grams less than that of L1L1 and L1L2. There was no significance difference of the egg production of L2L2 layers from 25 to 30 week age. L2L2 eggs showed lower of egg albumen height and Haugh unit. Activity of lysozyme of L1L1 was higher than that of L1L2 and L2L2. This research hinted that L1 and L2 alleles of lysozyme should impact the layer performance with dominant and recessive genetic effects, L1 is dominant to L2. LYZC would be a candidate gene for selecting layer performance.

Keywords: layer, lysozyme, allele, performance

S2- 0093 Effects of eggshell ultrastructural organization on hatchability in two strains of laying hens

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Eggshell ultrastructural organization is an important eggshell quality parameter which associates with hatchability. The objective of this study was to get a comprehensive understanding of the effects of eggshell ultrastructural properties on hatchability in two strains. The results indicate that the Strain A had heavier eggshell weight and thicker shell thickness, mammillary layer thickness and effective layer thickness compared to Strain B. As such, the mammillary layer of Strain A has the smaller size of mammillary cones than Strain B ($P < 0.05$). Eggs of Strain A had significantly higher eggshell strength than Strain B one ($P < 0.01$). In addition, a significant difference in hatchability among eggs from two strains was found. Comparing to Strain B (90.1%), Strain A breed was associated with a lower hatchability of fertile eggs (82.1%), suggesting that these eggshell ultrastructural properties could great significance to hatchability.

Keywords: strain, hatchability, eggshell ultrastructural properties

S2- 0094 Association of EDNRB2 gene polymorphism with the plumage color phenotype in Muscovy duck

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Endothelin receptor B subtype 2 (EDNRB2) is a paralog of the EDNRB, encoding a seven-transmembrane G-protein coupled receptor. It is reported that EDNRB, including EDNRB2 is essential for melanoblast migration along the dorsolateral pathway in mouse and human, as well as in Aves. According to the plumage color, Muscovy ducks have several types, such as white and black. To identify the association of EDNRB polymorphism with the plumage color phenotype in Muscovy ducks, the 60 black and white Muscovy ducks were selected and EDNRB2 gene polymorphism was analyzed. The Muscovy duck EDNRB2 gene was cloned. The length of sequence and the coding sequence (CDS) of EDNRB2 were 6484bp, 1311bp respectively. EDNRB2 gene included 7 exons and 6 introns, encoding 437 amino acids. The coding region was screened and the potential single nucleotide polymorphism (SNPs) was identified between white Muscovy ducks and black Muscovy ducks. Eight mutations were identified, including one missense variant c.64C>T, resulted in the amino substitution of Cys>Arg, and 7 synonymous substitutions (c.273G>C, c.402C>T, c.636G>A and c.672C>T, c.858G>A, c.1077C>T and c.1227C>T). Meanwhile, the c.273G>C mutation was completely linked with c.402C>T mutation and these two mutations were related with plumage color phenotype ($P < 0.01$): all black Muscovy ducks were only CC genotype and white Muscovy ducks were three genotypes(CC,CT and TT). The results provided important insights about different phenotypic associations with EDNRB2 mutations in Muscovy ducks.

Keywords: Muscovy duck, EDNRB2, polymorphism

S2- 0095 Transcriptome profiling identifies differentially expressed genes associated with high rates of egg production in chicken

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As was known, breeding process cycle of laying hens was regulated by the hypothalamic-pituitary-gonadal (HPG) axis, the ovary would affect egg production in the whole laying period because of the various hormones generated by hypophysis during follicle developing, maturation and ovulation. To identify the key regulatory genes and pathways involved in high egg production (HEP) on HPG axis, a total of 856 Chinese Luhua chicken was raised in the poultry breeding farm of Sichuan Agricultural University, the highest two hundred and the lowest two hundred egg production chicken were considered as HEP and low egg production (LEP) according to the egg number at 300 days of age (EGG300), respectively. RNA from HPG axis tissues (hypophysis, pituitary gland and ovary) of three (HEP) and three (LEP) chickens were isolated and sequenced through RNA-seq sequencing according to the similar laying rhythm and the EGG300, respectively. Normalized FPKM values were used to determine the gene expression level, MEV and DAVID were used to perform comparison analysis and Functional enrichment analysis of Gene Ontology terms and KEGG pathways, respectively. A total 86.7 Gb of RNA-seq sequences were generated from 17 libraries with each library averaged 5.1 Gb. In total, 1335 target genes were identified, and 10 of those were found to be possibly related to HEP, they were TBX2, LAPTM4B, MBP existed in hypophysis; SARIA, TXN and TMEM47 existed in pituitary gland; PRL, SCG2, STMN2 and CYP11A1 existed in ovary. Pathways such as MAPK signaling pathway, calcium signaling pathway, dopaminergic synapse and oxytocin signaling pathway were found to be possibly related to HEP. As was known, although previous studies have explored the differentially expressed genes between HEP and LEP in chicken ovary, the differences on HPG axis have not been researched comprehensively. Thus, our study on HPG axis may throw new light on the genetic breeding and high egg production molecular marker selection.

Keywords: chicken, transcriptome, HPG axis, egg production

S2-0096 Distribution and differentiation of gut microbiota in the five breeds of pheasant

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Although the pheasants belong to the birds and also provide meat and egg products for humans like chickens, the heredity, breeding, feeding management, especially distribution and composition of the gut microbiota of pheasant are still unclear. In this study, using the next generation sequencing, we analyzed the fecal microbial metagenome of five pheasant breeds (n=62) (Chinese ring-necked pheasant, Mongolia pheasant, Phasianus versicolor, Phasianus colchicas and Melanistic Mutant Pheasant). The V4 hypervariable region of the 16S rDNA was amplified and sequenced by Illumina Miseq platform. Our results revealed that the dominant microorganisms of pheasant were same with chicken, while the proportion of Cyanobacteria was significantly higher in pheasant (11%) compare to chicken (0%) at phylum level. The diversity and abundance of gut microbiota of genus in pheasant were far more than chicken, furthermore, 182 genera were significantly different between pheasant and chicken. We inferred that the diversity and abundance of microorganisms may undertake important tasks in the performance of disease-resistant and meat production of pheasant. In addition, we detected the distribution and differentiation of the gut microbiota in five breeds of pheasant. Results shown that 75 genera were significantly different in pheasants. Among the 75 genera, most of genera were remarkably influenced by the breed of Chinese ring-necked pheasant, while Burkholderia and Roseburia which belong to phylum of Proteobacteria and Firmicutes was remarkably influenced by Phasianus versicolor. Here, we first time reported the composition and diversity of the gut microbiota in pheasant. These results suggested different genetic characteristics have different influence for the distribution and differentiation of gut microbiome which indicated that host and gut microbiota are coevolved.

Keywords: pheasant, metagenome, gut microbiota, distribution, differentiation

S2- 0097 Establishment and inheritance of gut microbiota in the chicken

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In vivipara, studies have shown that the initial gut microbiota of fetus are inherited from mother by placenta, amniotic fluid and birth canal, but the embryo of ovipara is an isolated unit and the establishment and inheritance of gut microbiota have not been quantitatively examined. Here, we performed metagenome sequencing on specimen of chick embryo, chick and hen, assembled gut microbial genomes, and revealed microbial characteristic to each phase during the developmental process of life. Chick embryo intestine samples (n=84) aged 4, 12 and 19 days and fecal sample series including hen at birth (n=13) and chick (n=113), aged 4, 21 and 42 days, were collected. Chicks had been maintained at the same location and feeding pattern which were identical with the hen. All experiments were conducted in the sterile environment by the same operator. In our study, microbes were able to be isolated from chick embryo. Four major phyla dominated the chick embryo gut microbiota: the Proteobacteria (89%) was the most predominant phylum, followed by Firmicutes, Actinobacteria and Bacteroidetes. However, the proportion of Firmicutes in the chick (76%) and hen (44%) were the most predominant phylum. Results suggested chick embryo was not completely sterile and *in vitro* environment was an important factor to influence the structure of microbiota. At genus level, the diversity of microbiota in chick embryo were more higher than those of chick and hen during the developmental process of life. In addition, *Halomonas* was the predominant bacteria genus in chick embryo and hen. Although there were no obvious genetic correlations of microbes between chick embryo and hen, one third of microbes in chick embryo was significantly similar to hen. Gut microbiota in chick embryo was firstly identified in the study. We compared the similarity and variability of microbes between embryo and hen which will provide a new insight for the interaction mechanism between host and gut microbiota.

Keywords: establishment, inheritance, gut microbiota, ovipara, chicken

S2-0098 Maternal dietary protein restrictions in broiler breeder hens enhance the performance of broiler offspring on both standard and low protein diets

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Maternal undernutrition can influence and program offspring performance, as already demonstrated in mammalian species. In contrast, far less is known about the effects of broiler breeder feed composition on the performance and health of their offspring. Therefore, an experiment was designed whereby successive generations of breeder hens (pure line A) received different levels of dietary protein. The F0 generation was divided in a control group receiving a standard breeder diet whereas the low protein group received a reduced balanced protein breeder diet (25% reduction in crude protein (CP)) throughout their entire lifespan. Their offspring, raised as F1 breeders, was again subdivided in a control and reduced CP group, resulting in 4 groups of breeder hens with different combinations of breeder diets. Male progeny of these F1 hens were raised as broilers according to standard guidelines until 6 weeks of age. These chickens were again divided in 2 groups, one with a standard broiler diet (C) the other with a low protein diet (LP) (15% of balanced CP reduction), resulting in 8 groups. Zootechnical performance of these 8 groups was registered weekly. Broilers, regardless the treatment of the (grand)parents, on C feed were heavier than broilers on LP feed from two weeks onwards and had a lower FCR. Furthermore, there were also effects originating from the feed in the F1 generation. Broilers on C feed originating from breeders receiving low protein diets in the F1 generation gained more weight (BW wk 6 = 3325 ± 23 g) than C groups originating from control fed F1 hens (BW wk 6 = 3167 ± 22 g). This effect was not observed for broilers on LP feed. Moreover, the FCR was lower in all broiler offspring origination from F1 breeders on LP breeder diets compared to offspring of F1 breeders that received a standard diet. In general, these results show that LP feed in the F1 breeders improves their broiler progeny performance, irrespective of the feed in the F0 generation.

Keywords: broiler breeders, offspring, low protein, maternal undernutrition, broiler performance

S2- 0099 Feeding broiler breeders a reduced balanced dietary protein level improves the total tract nitrogen digestibility of the broiler progeny

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Mammalian studies have shown that nutritional constraints during the perinatal period can program the progeny (metabolism, performance). This research aimed to investigate if the performance of the broiler progeny could be influenced by reduction of the crude protein (CP) level in the feed of breeders. These effects were investigated by reducing the dietary CP level with 25% during rearing and laying period of breeder hens (pure line A) in two consecutive generations. There were two groups in the F0-generation i.e. control (F0-C) and low protein (F0-LP) group. The F0-progeny of each group, raised as F1-breeders were either fed a C or LP diet, resulting in four groups; F1-C/C, F1-C/LP, F1-LP/C and F1-LP/LP (breeder feed in F0/F1 generation). Part of both the F0 and F1-progeny was also raised as broilers according to standard guidelines. The broiler progeny of F0-LP, F1-C/LP, and F1-LP/LP had a higher BW at slaughter age and showed a tendency towards a better FCR compared to the control groups F0-C and F1-C/C. Therefore, a digestibility trial was performed with the broiler progeny of the four F1 breeders groups. In week 3, the feces were collected for 4 consecutive days for nitrogen (N) analysis (Kjeldahl). The apparent fecal N digestibility of broilers originating from C/C, C/LP, LP/C and LP/LP F1-breeders was respectively $58.0 \pm 1.6\%$, $61.7 \pm 1\%$, $62.2 \pm 0.8\%$ and $64.0 \pm 0.9\%$. The N digestibility of broilers descending from the F0-LP hens was significantly higher ($p=0.03$) than that of their F0-C counterparts. It is not surprising that the effect of the maternal feed (C or LP) during the F1 generation was less pronounced ($p=0.06$), considering the transgenerational improvement on the N digestibility of broilers originating from F0-LP breeders. This phenomenon might be mediated by epigenetic mechanisms. These results indicate that the broiler performance and N digestibility can be improved by reducing the dietary CP level of the breeder hens.

Keywords: broiler breeders, maternal undernutrition, low protein, offspring, nitrogen digestibility

S2- 0100 The preliminary research progress on germplasm characteristics of Frizzled Feather Chicken (Kirin Chicken)

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Frizzled Feather Chicken (Kirin Chicken) is the local breed in Guangdong province of China. Its typical characteristics include yellow and frizzled feathers, and anti-heat stress. It is adapted to the subtropical regions. The paper is a preliminary study about growth performance, the feather development and molecular analysis of the breed. The results show that: 1. The growth performance: The plumage are yellow and frizzled of the breed. The body weight of 1-day-old was 34.7g and 33.9g of the male and female, respectively. 16-week-old body weight was 2365g and 1591g of the male and female respectively. And feed conversion ratio was 3.36 and 3.73 of the male and female respectively. The eviscerated yield with giblet was 84.9% and 83.4% of the male and female respectively. The abdominal fat rate was 1.8% and 3.5% of the male and female respectively. 2. The feather development: (1) The embryonic follicle characteristics: There were some differences in the development of the medulla and barb ridge between frizzled feather follicle and normal feather follicle from E12 to E15. At the E13 day, the medulla of frizzled feather were bigger than normal feather, but the barbule plates were smaller than normal feather. (2) The frizzled feather growing after hatching out of their shells: The back plumule of chicks appear a little bit of curling at 1 day of age. Wing feathers appear visible curling at 3 day of age. At 5 weeks of age old, the frizzled feathers will be all over their body. (3) The morphological characteristics of frizzled feather: We found that frizzled feather branchlets can not collude and close into a complete pinna by microscope. 3. The molecular analysis: the CDS of KRT75 gene was 1569 bp in Kirin chicken. And the gene expression of KRT75 was significantly difference between Kirin chickens and Princess chickens of the feather follicle in embryo. Three SNPs (955bp: T>C; 967bp: T>C; 978bp: C>T) were found compared Kirin chicken with Princess chicken.

Keywords: Frizzled Feather Chicken, Kirin chicken, germplasm characteristics, anti-heat stress, KRT75 gene

S2- 0101 Identification of gene expression changes upon acute heat stress exposure in broiler chickens using mRNA-sequencing

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Acute heat stress affects poultry production by regulating immune response. In the present study, we analyzed global gene expression changes in three tissues (leg muscle, thymus and bursa of Fabricius) of the white broiler chicken (*Gallus gallus*) after acute heat stress by using mRNA-sequencing (RNA-seq). The heat-stressed group was subjected to acute heat stress at 40 °C for 3 hours and the control group was maintained at 25 °C. As a result, we detected 125, 79 and 93 differentially expressed genes in leg muscle, thymus and bursa of Fabricius, respectively. These differentially expressed genes were subject to co-expression network analysis and gene ontology analysis. Our study provides new insights into the heat stress response in chickens.

Keywords: broiler chicken, acute heat stress, mRNA-sequencing

S2- 0105 Identification and analysis of antisense promoter regions of chicken GHR gene

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Chicken GHR antisense transcripts (cGHR-AS) were found existing in chicken liver using Digital Gene Expression Tag Profiling sequencing technology. Meanwhile, through the antisense strand specific PCR and sequencing method, we preliminary confirmed that cGHR-AS were expressed in chicken muscle and liver tissues, and may be a long non-coding RNA. Bioinformatics analysis showed that the transcription start site of the cGHR-AS located in upstream of 1.7 kb deletion sequence in sex-linked dwarf chicken. This study provides a foundation for further analysis of emerging mechanism of cGHR-AS. With online softwares Promoterscan and methprimer, we predicted the cGHR gene antisense promoter (ASP) region and CpG island area. We successfully constructed a series of cGHR-ASP luciferin enzyme report gene vectors with different length of cGHR-ASPs, and various mutation reconstructions of interested transcriptional factors binding sites. All reconstructions were performed transient transfection and dual-luciferase activity detection. Results show that there are three cGHR-ASPs named as ASP1-2915, ASP2-1673 and ASP3-1180, respectively and without potential CpG islands in cGHR-ASP region. The luciferase reporter gene assay showed that cGHR-ASPs exhibited high activity in chicken hepatocellular carcinoma cell line LMH, followed by chicken myoblast and DF-1 cells ($P < 0.05$ vs pGL3-basic). Relative to the position of cGHR mRNA transcription start site ATG, ASP1, ASP2 and ASP3 were located in the region of +57238 bp ~ +54333 bp, +75977 bp ~ +74303 bp and +77143 bp ~ +75964 bp, whose core region of were located in +55243 bp ~ +54333 bp, +74982 bp ~ +74303 bp and +76837 bp ~ +75964 bp, respectively. Transient transfection experiment indicated that ETF and PR transcription factors down-regulated the promoter activity of ASP1. And MyoD inhibited the promoter activity of ASP2, whereas CdxA1 and CdxA2 conjugate regulating the promoter activity of ASP3.

Keywords: chicken, GHR, antisense promoter, core region, transcriptional factor.

S2-0106 Microsatellite analysis of indigenous ducks of tamil nadu

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Tamil Nadu is the southernmost state in India, where duck farming is predominant. The two popular indigenous duck varieties of Tamil Nadu (Sanyasi and Keeri) have innate potential to produce considerably more number of eggs under nil input system of management. In addition, they also contribute to improving the nutritional status of the rural poor. The genetic structure of these duck varieties was assessed with 23 FAO recommended duck specific microsatellite markers using advanced automated genotyping technique. The analysis revealed that in Sanyasi variety, 136 alleles were observed with the number ranging from 3 (CAUD017, CAUD027, CAUD031, CAUD033 and APH001) to 19 (CAUD024) and an overall mean of 5.91 ± 0.76 across the loci. In Keeri variety, 129 alleles were observed with the number ranging from 3 (CAUD013, CAUD017, CAUD023, CAUD026, CAUD027 and CAUD031) to 15 (CAUD024) and an overall mean of 5.61 ± 0.66 across the loci. The mean observed and expected heterozygosities for Sanyasi ducks was 0.5196 and 0.5628, whereas for Keeri ducks, it was 0.5217 and 0.5258 respectively. All the microsatellite loci were found to be highly polymorphic. Sixty one per cent in Sanyasi and 52 per cent of loci in Keeri ducks had PIC values of more than 0.5 indicating that these markers can be effectively used for genetic diversity analysis. The Chi-square (χ^2) test revealed that among the 23 microsatellite studied, only 7 in Sanyasi and 8 in Keeri were in Hardy-Weinberg equilibrium proportions and the rest departed from equilibrium. Selection and non-random mating could be the main reasons for this disequilibrium. The markers used in the study were found to be highly informative, explores high genetic variation in the population which could be exploited for their improvement.

Keywords: microsatellite, Sanyasi, Keeri, indigenous ducks, Tamil Nadu, India

S2-0107 Identification of laying-related snps in three-yellow chicken

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Laying performance is an important economical trait in poultry production. It is very difficult to improve the genetic progress of traits of low heritability, such as laying performance, using traditional methods. Molecular marker is good strategy to assist the selection of individuals. In current study, 12 candidate genes with 41 SNPs were chosen according to SNPs information in GenBank. Time of Flight Mass Spectrometer was employed to detect the SNPs of candidate gene in a total of 189 laying hen. The results showed that 20 SNPs hadn't polymorphisms, 21 SNPs existed polymorphisms. The frequency of gene and genotype of 21 SNPs were calculated in a total of 189 laying hen. The results showed that there were significant difference in the frequency of gene and genotypes in 10 SNPs ($P < 0.05$). These SNPs were rs14461387 of BMP3 (bone morphogenetic protein 3), rs739210538 of ESR α (estrogen receptor α), rs14986825, rs14986829, and rs312295326 of GHR (growth hormone receptor), rs734292086 and rs741266681 of IGF-1 (Insulin-like growth factor 1), rs736387506 of VIPR1 (vasoactive intestinal peptide receptor 1), rs731970748 and rs740278298 of VIPR2 (vasoactive intestinal peptide receptor 1), respectively. The current results suggested that 10 SNPs of 6 genes might be a possible candidate markers or targets for marker-assisted selection of egg numbers in three-yellow chicken.

Keywords: Three-yellow chicken, laying performance, laying-related SNPs, molecular marker

S2-0108 Polymorphisms analysis of MC1R in Guangxi native chicken

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The melanocortin 1 receptor (MC1R) gene is associated with the plumage color variations in chicken. In order to explore the relationship between the polymorphisms of MC1R gene and the variation of plumage color in Guangxi native chicken breeds, in current study, we tested eight breed lines with different color, Xiayan chicken (yellow), Three-yellow chicken (yellow), Donglan-Wu chicken (black), Longsheng-Feng chicken (black and white), Lingshan chicken (partridge), Lingyun-Wu chicken (partridge), Nandan Yao chicken (partridge), and Nandan-Yao chicken (white), respectively. 48 males and 48 females were selected and pooled to male and female DNA pooling, respectively. The full sequence of MC1R gene was amplified by PCR and subsequently sequenced. The results showed that there were 8 MC1R SNPs in eight Guangxi native chicken breeds lines. These SNPs were located in 69, 212, 274, 376, 398, 427, 636, and 637 nucleotide positions, respectively. Xiayan chicken shared a haplotype with Three-yellow chicken, Lingshan chicken shared a haplotype with Lingyun-Wu chicken and partridge Nandan-Yao chicken, Donglan-Wu chicken and White Nandan-Yao chicken have own haplotypes, respectively, while, haplotype of Longsheng-Feng chicken is a heterozygote of haplotype of Donglan-Wu chicken and White Nanshan-Yao chicken. In summary, the current study will be used for designing proper breeding and conversation strategies for Guangxi native chicken breeds, also for developing a molecular marker in chicken breeding.

Keywords: Guangxi native chicken, plumage color, MC1R, SNP

S2-0109 Effect of strain on the relative growth of gastrointestinal tracts of four strains of broiler chickens

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The study was conducted to investigate the effect of strain on the live weight and relative growth of gastrointestinal tract (GIT) organs of broiler birds. The experiment was carried out with 400 day-old unsexed broiler chickens of 4 different strains. The birds were randomly allocated into 4 treatment groups according to the strains; ST1, ST2, ST3 and ST4. Each treatment comprises of 2 replicates with 50 birds per replicate, reared for 49 days (7 weeks) and fed the same diet. On day 21 and 49, two birds were taken from each replicate, weighed and sacrificed by cutting the jugular vein. The weights and lengths of visceral organs and segments of gastrointestinal tracts were taken and recorded. Data obtained were then subjected to analysis of variance. There was no significant strain differences ($P>0.05$) in the live weights and in the weights of different organs except for gizzard which was found to be least heavy in ST2 strain at 3rd week. Strain differences were also observed in the lengths of heart, proventriculus, crop and gizzard, while other organs remained the same across the strain at day 21. There were significant strain differences ($P>0.05$) for gizzard, duodenum and ceca weights in proportion to live weight at day 49. Gizzard and duodenum were bigger in ST1 than in other strains. However, live weight, heart, crop, proventriculus, liver, pancreas, jejunum, ileum and colon weights were not influenced ($P>0.05$) by strain differences. Organ lengths were also influenced by strain differences with most obvious differences ($P>0.05$) found in the duodenum length of ST1. Crop, pancreas and colon differed ($P>0.05$) among the strains in lengths at day 49. It was concluded that differences exist only in the GIT organs but not in the live weight. ST1, ST2, ST3 and ST4 broilers have equal genetic potentials for growth despite differences observed in their GIT development.

Keywords: gastrointestinal tract, broiler strain, gut morphology

S2-0110 The effect of diet inclusion of Tributyrin in broiler breeders on egg quality and the performance of their day-old chicks

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Butyric acid is well-known for its biological functions in animals and human. The aim of the study is to evaluate the impact of diet supplementation of butyric acid in the form of Tributyrin on egg quality, fertility, hatching rate and the performance of their day-old chicks during the first 8 days of life. The trial is run on female D line Hubbard breeds. The performance of breeders in three treatment groups is compared: Group 1 = Control, standard diet; Group 2 = Control + Tributyrin (ProPhorceTM SR 130 at 0.5 kg/ton); Group 3 = Control + Tributyrin (ProPhorceTM SR 130 at 1.0 kg/ton). Breeder performance in the farm (mortality, daily feed consumption and laying rate) is recorded between 49 and 56 weeks of age. Egg quality measurements (individual egg weight, shell static stiffness, shell fracture force and Haugh Units) are performed at 2 different ages: 48 and 56 weeks of age. Hatchability and day-old chick performance are measured at 56 weeks of age. SAS software is used to run statistical tests. The results on the egg quality traits did not show statistical significant differences between birds fed control diet or Tributyrin containing diet. The results of the Hatching test indicated statistical significant differences in fertility rate ($p=0.004$) resp 82.1% (Group 1), 84.5% (Group 2) and 85.3% (group 3) and Hatching rate ($p=0.011$) resp 75% (Group 1), 77.8% (Group 2) and 78.2% (group 3) between the groups receiving the control diet or diet supplemented with Tributyrin. Day- Old chick (DOC) performance resulted in statistical significant differences measured by the relative growth rate at 8 days of age; ($P=0.030$) resp. 200.2% (DOC of Group 1), 212.4% (DOC of Group 2) and 209.6% (DOC of Group 3). It can be concluded that the diet supplementation of Tributyrin had statistical significant effects on egg fertility, hatching rate and DOC growth rate during the first week of life. However egg quality remained similar in all groups.

Keywords: Breeders, day-old chick, tributyrin, fertility, hatchability

S2-0112 Positive regulation of chicken hepatic miR- 122 expression by HNF3 β

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MicroRNAs are a class of small noncoding RNAs about 22 nt in length that can target mRNA via base pairing to cause translational repression or mRNA degradation. miR-122 is the most abundant microRNA in the chicken liver and regulates metabolic pathways including cholesterol biosynthesis, fatty acid synthesis and oxidation. In order to know the reason of the abundant expression of hepatic miR-122 in chicken, the regulatory region of miR-122 gene was analyzed in present study. Bioinformatics analysis and 5' -RACE were performed to analyze the primary transcript structure, promoter region, and potential transacting factor binding sites of miR-122 gene in chicken. Reporter gene assays integrated with truncation and site-mutation in miR-122 promoter were performed to determine the trans-activation effect of HNF3 β to miR-122-promoter in vitro. EMSA assays were performed to verify HNF3 β binding to miR-122 promoter. It was predicted that the chicken miR-122 gene promoter was located in 3.8~3.2 kb upstream region of pre-miR-122, and its transcription start site was found at the 3294bp position upstream (chrZ:757760) of pre-miR-122. A potential HNF3 β binding site was predicated at upstream of initiation transcription site. In cotransfection experiments, this site mutated would significantly decrease the luciferase activity in LMH, indicating it plays an important role for miR-122 transcription. The further experiment of the HNF3 β over expression plasmid pcDNA3.1-HNF3 β and pGL3b-p-494 vector being co-transfected into LMH cell line certified that HNF3 β could active miR-122 transcription. The EMSA experiment proved that HNF3 β can bind to the promoter region of chicken miR-122 in vitro, suggesting that HNF3 β can bind to the site to activate miR-122 transcription. These data together indicated that miR-122 expression positively regulated by the liver specific transcription factor HNF3 β might be one of the reasons for the high expression of miR-122 in chicken liver.

Keywords: Chicken, miR- 122, HNF3 β , gene expression regulation

S2-0113 Microsatellite marker analysis for the genetic relationships among six guangxi chicken breeds and two commercial breeds

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Guangxi Zhuang Autonomous Region has a wide variety of local chickens. A better understanding of genetic diversity and structure of Guangxi local chicken will provide baseline data for the evaluation and improvement of current breed conservation. The aim of this study was to examine the population genetics and structure of six Guangxi local breeds. A total of 280 birds including 240 six Guangxi local chicken breeds (LS: 45, ND: 45, GX: 40, TY:35, MA:40, XY: 35), 20 Arbor Acres chicken (AA), and 20 Roman chicken (RM) were selected in the current study. Genetic DNA was extracted from blood for microsatellite analysis using 18 markers (MCW0123, ADL0112, MCW0014, MCW0034, MCW0103, MCW0295, MCW0078, MCW0222, MCW0098, MCW0111, MCW0037, MCW0248, LEI0116, ADL0268, MCW0216, MCW0020, MCW0206, MCW0183). The results showed that 139 alleles were detected at 18 microsatellite loci in the 280 birds from the six native Guangxi chicken breeds and two commercial breeds. The average number of alleles was 7.72. The HO and HE ranged from 0.3630 to 0.8423. The PIC of loci was 0.6234. The phylogenetic tree of the six Guangxi native chicken breeds and two commercial breeds was constructed based on Nei' distance, using the UPGMA method. Two commercial breeds, AA and RM, were found to be clustered together but were found to be segregated from the Guangxi breeds. Among the Guangxi breeds, the TY, XY and MA were grouped into the same branch while the ND and GX were grouped into another branch, with the LS belonging to a single separate branch. The current study suggests that Guangxi native breeds have a rich genetic diversity and these genetic information would provide very important information for the evaluation and improvement of current conservation strategies of Guangxi native chicken.

Keywords: Guangxi, chicken, microsatellite markers, genetic diversity

S2-0114 Cryopreservation and characteristics of traditional old chicken breeds in Germany

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Before industrialization, many different chicken breeds were used in agriculture. Today, in commercial breeding there are only a few specialized chicken lines used in hybridization programmes. In Europe, the majority of biodiversity is still kept by hobby breeders. Many old breeds are at risk of extinction because of small population sizes and a lack of systematic conservation programmes. In addition, it is unknown if these old breeds still show their past production characteristics, for example in terms of laying performance. As an important element of the National gene bank a cryopreservation project of cock sperm has been launched for 12 traditional German breeds. Selection of cocks hatched for cryopreservation is based on molecular and phenotypic data. Twenty nine microsatellites were used to rank cocks according to their calculated relative contribution to a core set of individuals displaying maximal diversity within each breed. Phenotypes were evaluated by judges according to breed standards. On average 15 selected cocks per breed served as sperm donors. The semen quality of the individual cocks was examined both before and after freezing by means of motility measuring (CASA system) and living / dead staining (FACS). The freezing-thawing process had a harmful effect on sperm motility and viability in all breeds. The viability decreased rather uniformly in all breeds to approx. 50% of the initial value. In the frozen-thawed samples, the amount of live cells varied from 45.5% to 60%. The important quality criterion of progressive motile sperm, however, showed considerably different reductions between the breeds. Frozen-thawed sperm motility was found to range from 19% to 39%. Laying performance of the hens and egg quality were also recorded. Laying only reached 59% to 78% of the breed standard. These findings show clearly that hobby breeding is more focused on the phenotype rather than on performance parameters.

Keywords: biodiversity, cryopreservation, cock sperm, production characteristics, laying performance

S2- 0115 Comparative morphometric traits in four varieties of Naked Neck chicken in Pakistan

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Present study evaluated a comparison of morphometric traits among four varieties of Naked Neck chicken. A total of 320 birds were arranged according to completely randomized design and divided into 4 treatment groups having 5 replicates of 16 birds each. Treatment consisted four varieties of Naked Neck chicken i.e., black, black-white, light brown and dark brown. Morphological traits were recorded at the age of 8 weeks. Light brown variety showed higher neck length and drumstick circumference as compared to other varieties whereas dark brown variety revealed higher keel and shank length, shank circumference and wing spread. However, no differences were observed regarding body weight, drumstick circumference and body length. It is concluded that variation exists regarding morphometric traits among four varieties of Naked Neck chicken located in Pakistan.

Keywords: Naked neck, varieties, morphometric traits, Pakistan

S2-0116 Effect of genotype and genotype–environment interaction on productive performance of Japanese quail varieties

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Two experiments were conducted in poultry farm of Department of Animal Resources – College of Agriculture – University of Diyala / Iraq, one experiment in autumn using 324 birds and the second in spring season using 397 birds reared for ten weeks, the study aimed to detect the performance of three varieties of Japanese quail(white, black and brown plumage color) in natural condition of spring and autumn seasons in Iraq and the effect of genotype \times environment interaction ($G \times E$) on meat and egg production. The experimental design was a factorial experiment in completely randomized design (3×2) with three replicates. The results showed the significant superiority of black variety in body weight during the first 5 weeks of age while there were no significant differences found in body weight between black and brown during 6 and 7 weeks of age. There were significant differences due to $G \times E$ in body weight in all weeks of rearing. The results recorded significant superiority of black variety on white variety in food consumption and weight gain, and also, there was the significant superiority of spring season rearing on autumn rearing in respect of weight gain and food conversion, also there were significant $G \times E$ in food conversion. There was the significant superiority of autumn rearing in the age of sexual maturity of females, hen day egg production (%) and a number of eggs per hen.

Keywords: Japanese quail, varieties, season effects, genotype - environment interaction, meat production

S2- 0117 Autosomal dwarfism and egg production performance of fowl

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Partial diallel crossing of Rhode Island Red (RIR), White Leghorn (WLH), Fayoumi (FO), Deshi (local) normal (DN) and Deshi dwarf (DD) chicken produced RIR, WLH, FO, DN, DD, RIR×DD, WLH×DD and FO×DD progenies. The crossbreds were separated into normal and dwarf at 19 weeks of age. Thus 11 genetic groups; RIR, WLH, FO, DN, DD, RIR×DD normal, RIR×DD dwarf, WLH×DD normal, WLH×DD dwarf, FO×DD normal and FO×DD dwarf. Between 19 and 46 weeks of age, 109 pullets belonging to 11 genetic groups were individually caged. Age and their egg production performance was compared. Introgression of adw gene to different breeds reduced ($P<0.01$) mature body weights and feed intake. As a result, adw pullets were more efficient in converting feed into egg mass in comparison with their normal size counterparts. Conservation and improvement of deshi dwarf chicken of Bangladesh might be use in future breeding to improve egg production.

Keywords: dwarf gene, body weight, egg production

S2-0118 Monitoring conservation effect on Chinese indigenous chicken breeds using MHC and DNA barcod

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We compared the Equimultiple Random Matching Pedigrees Conservation (ERMPC) and System Conservation (SCM) programs over three pedigree generations by measuring changes at the major histocompatibility complex (MHC) and certain phenotypic traits. Also conducted were computer simulations (CS). Egg shell, feather, and shank colors were consistent across generations for both programs. Generation by mating program interactions for percent fertility, hatchability, and livability to d 21 was greater in the 1st and 2nd generation for the SCM than ERMPC program. Weight of sexual maturity and at d 482 was greater for ERMPC than SCM in the 2nd generation. The increment of percent fertility, hatchability, and livability to d 21 was greater for SCM than ERMPC in each generation. From PCR-SCCP and sequencing analysis for MHC B-G with the 9 PCR products, the number of respective SNPs were 5, 6, 6, 6, 6, 6, 4, 9 and 7. Average heterozygosity (He) for the 9 MHC B-G products was 0.5094 and 0.5085 for the ERMPC and SCM programs, respectively. Polymorphic information content (PIC) of the 9 MHC B-G products was 0.6311 and 0.6194 for ERMPC and SCM, respectively. Although allelic frequencies across generations for SCM did not change significantly, some loci with relatively lower allelic frequencies were lost, which may have been due to drift as well as selection for production traits. According to the actual population size, sex ratios, and initial allelic frequencies, CS for 100 generations predicted the probability of such losses for a breeding population of the size used in ERMPC and SCM.

Keywords: Conservation, chickens, MHC, SNP, SSCP, Simulation

S2- 0119 IRT1-regulated mRNAs in chicken hepatocytes

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An important theme of Sirtuin 1 (SIRT1) is its striking ability to influence key aspects of metabolism. However, much less is known about the role of SIRT1 in chicken liver. The differentially expressed genes (DEGs) would be found in chicken hepatocytes after SIRT1 being knocked down and the target genes of SIRT1 associated with the hepatic metabolism would be revealed in this study. Primary hepatocytes were isolated from two-week chicken using an improved two-step collagenase method. Hepatocytes were cultured at a density of 1.2×10^7 cells in 10 mL William's E medium supplemented with 5% chicken serum, 100 U/mL penicillin - streptomycin, 10 μ g/mL insulin and 30 mmol/L NaCl at 37 °C with 5% CO₂. After being cultured 24 h, the primary hepatocytes were transfected with 145 pmol siRNA-sirt1 or control. Forty-eight h later, the total RNA from these hepatocytes was extracted to analyze the DEGs. Three reduplicate biological samples in which SIRT1 was knocked down were performed for RNA sequencing (RNA-seq) using Illumina NextSeq 500 by the Shanghai Personalbio Corporation. The RNA-seq results showed over 99% reads were clean reads and more than 95% reads were useful in the raw reads. 73.51%~79.20% clean reads could map to the chicken genome and uniquely mapped reads were less than 97.49%. 15,508 genes were identified from the mapped reads. A total of 86 DEGs were identified in hepatocytes between SIRT1 knocked down and control, of which 63 genes were down-regulated (≤ 0.5 , $P < 0.05$) and 23 genes were up-regulated (≥ 2 , $P < 0.05$). From the GO analysis, 60, 57 and 76 DEGs among them were related with biological process, cellular component and molecular function, respectively.

Keywords: SIRT1, DEGs, Primary chicken hepatocytes, RNA-seq

S2- 0120 Transcriptome sequencing to analyze molecular mechanisms underlying duck egg color

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Eggshell color is an important trait which influences hatchability, shell quality and consumer shopping. However, limited knowledge is available for molecular mechanisms underlying eggshell color. Jinding duck (JD) is a breed mainly laying blue eggs, in contrast, Beijing duck (BG) all lays white eggs. Here, transcriptomes from shell glands of JD (n=3) and BJ (n=3) were sequenced on Illumina HiSeq 2500 platform. After removal of low-quality sequences, we obtained a total of 198 million valid reads, of which 51.3 % were successfully mapped to duck reference genome and assembled into 19466 unigenes. Differential expression gene (DEG) analysis showed that expression of 1438 unigenes had significant difference ($P < 0.05$) between JD and BG, of which expression of 677 unigenes was up-regulated related to the BG. Gene Ontology (GO) terms analysis further reveals that most DEG were enriched into the cell component category, among which membrane, cytoplasm, membrane part, plasma membrane, signal transduction, integral component of membrane were the most significantly enriched GO terms. In conclusion, a large set of DEG between ducks laying blue and white eggs are found, which provides valuable information to further understand molecular mechanisms underlying duck eggshell color. GO terms analysis suggest that genes implicated in structure and function of membrane are very promising candidates for future identification of causative mutations.

Keywords: duck, eggshell color, transcriptome sequencing, differential expression gene, GO term

S2-0121 Association of VLDLR haplotypes with egg production supporting that VLDLR is a promising candidate gene for egg production

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VLDLR functions in transporting yolk precursors into the oocytes. However, knowledge gaps exist in distribution patterns of VLDLR variants among breeds and their association with egg production. Here, eight SNPs capturing 87 % of all VLDLR variants were genotyped in Nick Chick (NC, n=91), Lohmann Brown (LohB, n=50) and a Chinese indigenous breed of Lueyang chicken (LY, n=381). Egg production of the NC and LY were recorded from 17 to 50 weeks. Only four similar haplotypes were found in the NC and LohB, of which two main haplotypes accounted for 100 % of all NC haplotypes and 92.5 % of LohB haplotypes. However, considerable haplotypic diversity was maintained in the LY. Comparison of egg production within the LY showed that birds with NC-like haplotypes had significant higher production peak ($P < 0.05$) than ones without the haplotypes. However, VLDLR expression had no significant difference between different haplotypes ($P > 0.05$). Our study reported a discrepancy in distribution of VLDLR haplotypes between selected and non-selected breeds and suggested that the near fixation of VLDLR variants in the NC and LohB is compatible with signature of selection, which therefore adds new support on the VLDLR as a promising candidate gene for egg production.

Keywords: chicken, egg production, VLDLR, haplotype

S2-0122 Associations of malate dehydrogenase gene and uncoupling protein gene polymorphisms with fat deposition in commercial broilers

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This study aimed to evaluate the effects of single nucleotide polymorphisms (SNPs) in candidate genes on abdominal fat traits in chickens. A total of 376 Arbor Acres individuals are used in this study. The abdominal fat weight (AFW) and the abdominal fat percentage (AFP) were measured after slaughter at 7 weeks of age. All birds were genotyped for 32 SNPs distributed in 21 different candidate genes using Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) method. Association analysis showed that Malate Dehydrogenase (MD) gene SNP g.235 T>C and Uncoupling Protein (UCP) gene SNP c.1191 G>A were significantly associated with AFW and AFP ($P < 0.05$). For the g.235 T>C MD SNP, the birds with CT and CC genotype had significantly higher AFW and AFP than the birds with TT genotype ($P < 0.05$). For the c.1191 G>A UCP SNP, the birds with GG and GT genotype had significantly higher AFW and AFP than the birds with TT genotype ($P < 0.05$). Malate dehydrogenase (MD) is a key enzyme that plays an important role in energy metabolism. It was previously reported that the g.235 T>C MD SNP were significantly associated with AFW and AFP in the 8th generation populations of Northeast Agricultural University broiler lines divergently selected for abdominal fat (NEAUHLF). Uncoupling Protein (UCP) plays an important role in mitochondrial ATP metabolism and thermogenesis. Previous research found that the UCP gene SNP c.1197 A>C in strong linkage disequilibrium (LD) with the UCP gene SNP c.1191 G>A were significantly associated with AFW and AFP in the 5th generation population of NEAUHLF. It is concluded that the MD gene SNP g.235 T>C and the UCP gene SNP c.1191 G>A could be important molecular markers for fat deposition in chickens.

Keywords: Chicken, candidate gene, polymorphism, fatness

S2- 0123 Identification of chicken miR- 17- 5p and miR- 20a targeting LRIG1 gene

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MicroRNAs (miRNAs) are critical posttranscriptional regulators of gene expression and play vital roles in a wide variety of biological and pathological processes. Our previous study showed that overexpression of miR- 17- 92 cluster promotes chicken preadipocyte proliferation; however, the underlying mechanism is unclear. LRIG1 (leucine- rich repeats and immunoglobulin- like domains 1) is a tumor suppressor. Bioinformatics analysis by miRanda, TargetScan and PicTar software showed that LRIG1 was one of potential target genes of miR- 17- 5p and miR- 20a, which belong to miR- 17- 92 cluster. The objective of the present study was to identify whether miR- 17- 5p and miR- 20a target LRIG1 mRNA. We RT- PCR amplified the 3'UTR of LRIG1 mRNA, inserted into psi- CHECK2 to yield LRIG1 3'UTR reporter. After confirmation by double enzyme digestion and sequencing, psi- CHECK2- LRIG1 was cotransfected with 50 nM of miR- 17- 5p inhibitor, miR- 19a inhibitor and miR- 20a inhibitor into DF1 cells, respectively. After 48 h, Dual- Glow luciferase activity was detected. The reporter gene assay showed that, compared with negative control and miRNA- 19a inhibitor, miRNA- 17- 5p inhibitor and miR- 20a inhibitor were able to significantly increase luciferase reporter activity of psi- CHECK2- LRIG1 ($P < 0.05$). Our results demonstrated that LRIG1 is a specific target of miR- 17- 5p and miR- 20a.

Keywords: miR- 17- 5p, miR- 20a, LRIG1, chicken

S2-0124 Closing and function analysis of the genomic sequence gap upstream of chicken miR-17-92 cluster

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Although the transcriptional regulation of miR- 17- 92 cluster has been well studied in mammals, very little is known from chicken. There is a genomic sequence gap upstream of chicken miR- 17- 92 cluster. To date, the promoter location and sequence of chicken miR- 17- 92 cluster are unknown. In the present study, we closed the genomic sequence gap upstream chicken miR- 17- 92 cluster by chromosome walking. Sequence analysis showed that the genomic gap upstream of chicken miR- 17- 92 cluster was 1704 bp long, and its GC content was 80.11%. Sequence comparison among ten species (chicken, human, chimpanzee, cow, pig, rat, mouse, opossum, zebra fish and frog) showed there was a highly- conserved region, which is located in the closed gap, and corresponds to human miR- 17- 92 cluster promoter. Mulan software analysis showed that the conserved region has a number of conserved cis- regulatory elements including TATA box, initiator and myc binding site. We cloned the conserved region of the closed genomic sequence gap, and constructed its luciferase reporter pGL3- basic- miR- 17- 92 cluster. DF1 cells were cultured and transfected with pGL3- basic- miR- 17- 92 cluster, 48 h after transfection, luciferase reporter gene activity was measured. The reporter assay showed that the luciferase reporter activity of pGL3- basic- miR- 17- 92 cluster was more than 400 times greater than that of pGL3 basic empty vector. Taken together, Our results demonstrated that chicken miR- 17- 92 gene cluster promoter is located in the closed genomic gap region. Our results pave the way for revealing the transcriptional regulatory mechanisms of chicken miR- 17- 92 cluster.

Keywords: chicken, miR- 17- 92 cluster, transcriptional control, promoter

S2-0125 The identification of miR-17-92 cluster targeting ELK3 gene in chicken

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MiR-17-92 cluster is extensively studied in mammals and implicated in a wide variety of biological and pathological processes. However, little is known about the role and mechanism of miR-17-92 cluster in chicken. ELK3 is a member of the ETS family of transcription factors that links MAPK kinase signalling to transcription. Bioinformatics analysis showed that chicken ELK3 gene is a potential target of miR-17-5p, miR-19a and miR-19b which belong to miR-17-92 cluster. The objective of the present study was to identify whether ELK3 is a target gene of miR-17-92 cluster. We RT-PCR amplified the 3'UTR of ELK3 and cloned into the psi-CHECK2 dual reporter vector (psi-CHECK2-ELK3). After confirmation by double enzyme digestion and sequencing, psi-CHECK2-ELK3 was cotransfected with 50nM of miR-17-5p inhibitor, miR-19a inhibitor and miR-19b inhibitor, respectively, into DF1 cells. After 48 h, Dual-Glow luciferase activity was detected. The reporter gene assay showed that, compared with negative control, miRNA-17-5p inhibitor, miRNA-19a inhibitor, and miRNA-19b inhibitor were able to significantly increase luciferase activity of psi-CHECK2-ELK3 ($P < 0.01$). Our results demonstrated that ELK3 is target of miR-17-5p, miR-19a and miR-19b.

Keywords: chicken, miR-17-92 cluster, ELK3

S2-0126 Accuracy of genomic selection in a Chinese broiler chicken population

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Genomic selection (GS, Meuwissen et al, 2001) is a quick developing livestock breeding strategy. It is normally used in dairy cattle breeding, and attractive for pig and chicken breeding. The objective of this experiment is to evaluate the performance of GS on growth and carcass traits in a Chinese indigenous broiler chicken population. The population composed of 435 birds from 30 sire families in Wens Nanfang Poultry Breeding Co. Ltd (Xinxing, P.R. China). They were genotyped by Affymetrix 600K array and systematically phenotyped for 18 growth and carcass traits. After quality control, 468,020 SNPs and 435 birds were remained for further analyze. Ten folds cross validation and three prediction methods were used to perform GS in this population. The three methods are conventional BLUP, GBLUP (VanRaden, 2009), and BLUP|GA (Zhang et al, 2014). Variance components were also estimated for all traits using DMU software (Madsen et al, 2006). Accuracy of GS was defined as the correlation coefficient between predicted and true phenotypic values. Results showed that the heritability of these traits ranged from 0.28 to 0.60 with mean 0.40 estimated with pedigree, and from 0.24 to 0.52 with mean 0.42 estimated with genotypes. Mean accuracy over all traits were 0.29, 0.31 and 0.32, for BLUP, GBLUP, and BLUP|GA, respectively. Genomic selection showed advantage in prediction accuracy over conventional BLUP for 15 out of the 18 traits, BLUP|GA showed advantage over GBLUP for all traits analyzed. Accuracy increased with trait heritability for all traits and all prediction methods. We can conclude that it is potent to implement GS in such chicken population, especially for traits that is conventionally less improved, such as carcass traits. In the circumstance investigated, model selection is important for finally performance of a GS program. BLUP|GA which can take trait specific information into the prediction model is a method of choice with high density SNP data.

Keywords: genomic selection, broiler, growth traits, carcass traits, SNP

S2- 0127 DNA methylation-mediated transcription factors regulates Piwi1 expression during chicken spermatogenesis

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PIWI (P-element induced wimpy testis) protein family is responsible for initiating spermatogenesis and maintaining the integrity of germ cells and stem cells, but little information could be found describing its essential transcriptional regulation in poultry so far. By direct bisulfite pyrosequencing assay and transcription factors binding site prediction, we characterized the methylation status of chicken Piwi1 promoter region in five different spermatogenic cells which revealed a negative correlation with germ cell type-specific expression patterns of piwi1. -148 CpG site, as the transcription factor TCF3 and NRF1 binding site, was differentially methylated in different spermatogenic cells, which were completely methylated in PGCs, but unmethylated in round spermatids. The similar result was found in the posterior fraction of Piwi1 promoter CpG islands, SOX2 binding sites. In addition, demethylation assay further proved that DNA methylation indeed regulate Piwi1 expression during chicken spermatogenesis through activating corresponding transcription factors binding. Collectively, we reveal that spatio-temporal expression of chicken Piwi1 from PGCs to round spermatids is controlled by methylation-mediated transcription factor regulation.

Keywords: chicken, DNA methylation, piwi1, spermatogenesis

S2- 0129 A phenome database designed and constructed for Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHL)

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To effectively and efficiently curate and manage the large amounts of phenotypic and genotypic records collected during the divergent selection for abdominal fat content for 20 years at Northeast Agricultural University, the NEAUHLF Chicken Phenome Database (NCPD) was built as the coordination center for data management and analysis. The design principle and main objective of NCPD were to effectively curate and manage the large amounts of pedigree relationship and phenotypic records using the standard software, MySQL, and to efficiently retrieve data in appropriate formats for direct estimation of genetic parameters in MTDFREML, ASREML, etc., which integrated quantitative phenotype into a common annotated framework to facilitate query and analysis. Detailed records in NCPD includes 1) pedigree relationship for all broilers; 2) 29 phenotypic measurements or traits relevant to chicken growth and body composition, including reproductive, growth and carcass performance, at different growth stages for 20 generations; 3) genetic marker information of over 50 candidate genes examined for their relationship with the phenotypic records. Therefore, NCPD houses a wealth of line/strain characteristics and phenotypic data for studying chicken adiposity and gene function and testing hypotheses, to facilitate the use of chicken in both laboratory and field research. In the coming years, NCPD will maintain a growing collection of standardized reference phenotypic and genotypic data, to assist in selecting chicken lines/strains for better research applications.

Keywords: broiler, divergent selection, phenome, database

S2- 0130 Transcriptome and DNA methylome sequencing reveals the physiologic function of ceca in lines of chickens divergently selected for body weight

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The anatomy of the chicken ceca is quite different from mammals. The chicken ceca harbor large amounts of microbial communities. Here, we used RNA-Seq and MBD-Seq to investigate profiles of transcriptome and DNA methylome in cecal tissue, to study the functions and mechanisms of epigenetic regulation. The ceca tissue samples (n=20) were obtained from two lines of chickens which originated from a common founder population and had undergone >54 generations of selection for high (HW) or low (LW) 56-day body weight. The lines now differ > 10-fold in body weight at selection age. In our results, a total of 330 genes were found to be differentially expressed between the HW and LW lines, of which 201 genes were up-regulated and 129 genes were down-regulated in the HW compared to the LW line. GO enrichment analysis of differentially expressed genes (DEGs) revealed that they were enriched in cardiac muscle, including Myl2, ACTBL2, and CASQ2 and were all up-regulated in the HW line. In the muscle system process, which included MYBPC3, TNNC2, and ACTN2, only MYBPC3 and TNNC2 were up-regulated in the HW line. In the defense response, including GAL2, GAL6, and GAL7, and all were up-regulated in the LW line. We also detected 4779 different methylation regions (DMR) through whole chicken genome. There were 3430 hypermethylated DMRs and 1349 hypomethylated DMRs in HW compared to LW line. Among the DMRs, 641 different methylation genes (DMGs) were identified between the two lines. Moreover, integrated analysis uncovered 16 DEGs nested in other DMGs. SMAD1 was up-regulated and hypermethylated in HW line coding regions. Our results suggest that the ceca may play a significant role in formation of heart muscle. Since GAL2, GAL6, and GAL7 are genes which belong to the defense response and are up-regulated in the LW line, which suggests that the ceca may be an immune organ participating in innate immunity.

Keywords: transcriptome, DNA methylome, ceca, divergent selection, body weight, chickens

S2-0131 CRISPR/Cas9-mediated genome editing in chicken cells

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Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated (CRISPR/Cas9) system is the powerful tool for genome editing in several organisms. Genome editing in chicken mediated by CRISPR/Cas9 system has been expected to increase the value of chicken as a model animal, however, there have been few researches about genome editing by CRISPR/Cas9 system in avian species. Therefore, here, we performed CRISPR/Cas9 system mediated genome editing in chicken. As results, we identified gene mutations on targeted locus of DF1 cell lines by introduction of sequence specific guide RNA (gRNA) and Cas9 plasmid, and the efficiency was vary depending on target sequences. Confirmed gRNA and Cas9 were also introduced to chicken primordial germ cells (PGCs), these also successfully induced mutations on targeted locus of chicken PGCs. Furthermore, we performed targeted gene insertions on chicken DF1 cell lines and PGCs by CRISPR/Cas9 system. The integration was confirmed by genomic DNA analysis. The results suggest that CRISPR/Cas9 system could induce targeted genome editing in chicken cells and apply to establish novel model animals as well as studying germ cell biology.

Keywords: CRISPR/Cas9, chicken, genome editing

S2- 0132 Transcriptional regulation of chicken Perilipin1 by Retinoid X Receptor α

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Our previous study showed that the chicken RXR α could activate Perilipin1 gene promoter activities in DF1 cells and chicken primary preadipocytes. The objective of present study was to investigate whether Perilipin1 is regulated by RXR α in chickens. A series of different length fragments upstream of the start codon of chicken Perilipin1 gene were amplified by PCR and cloned into pGL3 basic vector to generate serial promoter reporter constructs. To detect the effect of RXR α on Perilipin1 promoter activity, the RXR α expression plasmid (pCMV-HA-RXR α) and the designated promoter reporter constructs were co-transfected into DF1 cells; The luciferase reporter assay was carried out according to the manufacturer's instructions (Promega); The bioinformatics software (Jaspar) was used to predict the transcriptional factor binding sites; EMSA and Site-directed mutagenesis were used to confirm whether RXR α specifically binds to the predicted binding site. The bioinformatics analysis showed that there was a potential RXR α binding site in the -774/-762 region of the chicken Perilipin1 promoter. The luciferase reporter assays showed that transfection of pCMV-HA-RXR α increased the reporter activity of all Perilipin1 promoter reporters compared with the negative control. Of these Perilipin1 promoter reporters, pGL3-Plin-830/-11 had the highest reporter activity and pGL3-Plin-680/-11 had the lowest activity. EMSA and mutational analysis revealed that the chicken RXR α could directly bind to the -774/-762 region and regulate the Perilipin1 promoter activity. These results could be contributed to understand the molecular mechanism of transcriptional regulation of chicken Perilipin1 gene in adipocytes.

Keywords: chicken, Perilipin1 gene, RXR α , transcriptional regulation

S2- 0133 Identification of chicken miR-454-3p targeting PPAR γ gene

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The peroxisome proliferator-activated receptor gamma (PPAR γ), a member of the PPAR subfamily of nuclear hormone receptors, is the master regulator of adipogenesis and obesity. To date, no miRNAs have been reported to target chicken PPAR γ gene. In the present study, we cloned the 3'-untranslated regions (3'UTR) of chicken PPAR γ gene using 3'RACE. The miRNAs targeting chicken PPAR γ were predicted using online software TargetScan and validated using the dual luciferase reporter gene assays. TargetScan analysis predicted that the 3'UTR of chicken PPAR γ mRNA contains a miR-454-3p binding site. To test this predication, we constructed a wild-type PPAR γ 3'UTR reporter (psiCHECK-2-PPAR γ -WT) and a mutant reporter with a mutation in the predicted miR-454-3p binding site (psiCHECK-2-PPAR γ -MUT). The psiCHECK-2-PPAR γ -WT or psiCHECK-2-PPAR γ -MUT was cotransfected with miR-454-3p mimic into DF1 cells. After 48 h, Luciferase reporter activity was measured. The reporter gene assay showed miR-454-3p mimic reduced luciferase reporter activity of psiCHECK-2-PPAR γ -WT by 50%, compared with mimic negative control. In contrast, miR-454-3p mimic had no effect on reporter activity of psiCHECK-2-PPAR γ -MUT. Taken together, our results demonstrated that chicken PPAR γ is a direct target of miR-454-3p. Our results lay a foundation for unraveling the post-transcriptional regulation of PPAR γ gene in chicken.

Keywords: chicken, gga-miR-454-3p, PPAR γ , target gene

S2- 0134 A preliminary study on breeder's natural mating system of Yellow-feather Meat Chicken

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Animal husbandry shows a trend of becoming more mechanized, and more automatic. Sichuan Daheng Poultry Breeding Company first carried out the research about the effects of natural mating system by using the Yellow-feather Meat Chicken. This study will analyze focusing on the following aspects: equipment, feeding and management and ethology. We also combine the performance trait to estimate the natural mating system and proposed improvement measures. We innovatively designed a cage for broiler breeder, when compared with layer it's greater than 50% in area of per individual activity. As it is found in feeding experiment, the breeder should transfer to the mating cage before start laying egg, preferably 30 days to make them easier to feed-control and assure the breeder's development and sexual maturity. In order to establish the social rank order of cocks, it's better to load all males and females at the same time. The fertility rate and hatching rate reach to a peak value when the ratio of male:female is 1:8. The natural mating system is better because of avoiding artificial insemination and for its adaptation to animal natural behavior. The study also found that mixed feed of rooster earlier than sexually mature before too long, is not conducive to the feed control and weight control, can not guarantee the cocks' normal growth and development. Wu found when the male and female ratio is higher than 1:8, it will reduce the rate of egg fertilization and hatching rate and increase production costs because of the more males will increase the consumption of cock fighting. And if the ratio is lower, the hens can not be completely finished mating. It is important to improve the animal welfare by natural mating system, but much work is going on in this area.

Keywords: Yellow-feather meat chicken; broiler breeder; natural mating system; animal ethology; animal welfare

S2- 0135 Host immunocompetence alters the gut microbiota in lines of chickens divergently selected for antibody titers

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It has long been recognized that the gut microbiome is influenced by the host's immune system. Less is known on how host immunity regulates the gut microbiome. In this study we used lines of chickens divergently selected long-term from a common founder population for either high or low antibody response 5 days post-injection of a non-pathogenic antigen, sheep red blood cells (SRBC). The objective was to study host effects on the gut microbiome profile. Fecal samples were collected from 125 adult chickens, which included from generation 40 30 female and 13 male high antibody selected (HAS40) and 20 female and 8 male low antibody selected (LAS40), and from generation 17 17 female and 10 male high antibody relaxed (HAR17) and 19 female and 8 male low antibody relaxed (LAR17). To understand how host immunity shapes the gut microbiome and interacts with it to affect the host immunity response, we compared the abundance of microbiota taxa and their functional performance among these lines of chickens. There is >5-fold difference between the H and L selected lines in SRBC host immunity response. We observed that 13 genus show significant differences between the high and low antibody lines, with 2 genus significantly influenced by gender. More interesting, selection pressure different also significant influence 28 genus. Furthermore, the enrichment of microbiota function pathways implicated in infectious diseases, immune system conditions, endocrine effects, and metabolism of terpenoids and polyketides were significantly influenced in the low antibody lines. The results demonstrate that host immunity response has a pervasive role in regulating composition and diversity of gut microbiota. They support the hypothesis that host and gut microbiota coevolve, providing further evidence that host genetics influence the composition of the gut microbiome.

Keywords: coevolution, gut microbiota, immunity, divergent selection lines, chickens

S2-0136 Fast growing poses the risk of tarsal joint swelling in broilers

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Leg diseases, including tarsal joint swelling, have become a more and more severe disease in the modern broiler industry. Causing the detrimental effect of tarsal joint swelling can be attributed to multiple factors involving in genetic background of broilers. The aim of the current study was to understand the genetic effect on tarsal joint swelling and the relationship between tarsal joint swelling and growth traits in broilers. This study indicated the genetic factor was one of the most important causes resulting in the tarsal joint swelling of broilers. The estimated heritability of this trait was 0.27. The tarsal joint swelling was highly significantly associated with growth traits in broilers ($P < 0.01$). The broilers with more severe tarsal joint swelling had larger body weight. The genetic correlations between the tarsal joint swelling and body weights ranged from 0.44 to 0.63. The genome-wide association study identified that the region at 172-175 Mb on gallus gallus chromosome 1 had the most important quantitative trait locus for the tarsal joint swelling. Interestingly, this region was strongly consistent with the region controlling growth traits. Taken together, the same genes probably influenced body weights and the tarsal joint swelling in broilers, even the fast-growing broilers had the larger risk of tarsal joint swelling. Acknowledgments: This work was supported by National Key Technology Research and Development Program of the Ministry of Science and Technology of China (2012BAD39B04), the Pearl River Nova Program of Guangzhou (Grant No. 201506010017) and the Earmarked Fund for Modern Agro-Industry Technology Research System (Grant No. nycyt42).

Keywords: Tarsal joint swelling, body weight, genome-wide association study, broiler

S2-0137 Sire Influence on hatchability and heritability estimates of quails in a humid tropical environment

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Study on Japanese quail was undertaken to determine sire influence on hatchability and estimation of heritability values among three strains of the bird in a humid tropical environment in Nigeria. Fertility and hatchability traits; body weights (BW), Shank length (SL), thigh length (TL), Breast length (BL), body length (BL), keel length (KL), and Wing length (WL) at 2nd, 6th, 10th weeks of age, were measured on 300 crossbred progeny from 270 cinnamon brown dam, mated with three different strains of sire namely; cinnamon brown (CB), Panda white (PW), and Silver brown (SB) in the mating ratio of 1:4. The data were subjected to analysis of variance appropriate for completely randomized block design (CRBD), and significant means separated with Duncan's multiple range tests. Heritability estimate was done using the sire component equation. The average percent fertility (71.64% ~ 75.76%), hatchability (50.41% ~ 57.17%), percent dead in germ (11.20% ~ 18.25%), dead in shell (11.201% ~ 18.25%) were significantly ($P < 0.05$) better in PW-sired progeny, while pipped (13.06% ~ 20.11%), brooding (3.00% ~ 4.67%) and rearing (1.00% ~ 1.33%) mortality showed no significant difference ($P > 0.05$) among the three sired - progeny. The analysis showed that at week 2, 6, 10, SB-sired progenies had superior heritability estimates in most of the linear traits and recorded lower indeterminate values as compared with other sired - progenies. Moderate to high heritability estimates (42% ~ 83%) obtained for body weight at ages 6 and 10 weeks among the three progenies suggests that selection for body weight or growth rate in Japanese quail should be carried out at 6th and 10th weeks of age and that PW - sired progeny be selected for better hatchability for enhanced breeding program for both egg and meat production in the strains.

Keywords: Sire, hatchability, heritability, Quails and Humid tropics.

S2-0138 The roundabout, axon guidance receptor, homolog 2 gene contributes to immune responses to Newcastle disease virus by regulating the maturation of chicken dendritic cells

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The roundabout, axon guidance receptor, homolog 2 (ROBO2) as a member of the immunoglobulin superfamily can participate in neurodevelopment and immune responses in humans and animals. However, the understanding on immunologic function of ROBO2 gene is much less than that on neurobiological function of the gene. Our previous study had identified one single nucleotide polymorphism (SNP) in the chicken ROBO2 gene was associated with the antibody responses to Newcastle disease virus. The current study found two groups of chicken dendritic cells with the two opposite homozygous genotypes of the SNP had significantly differential expression of the ROBO2 gene at first. After 24 hours post-immunizing the LaSota strain of Newcastle disease virus, the two groups of chicken dendritic cells were going maturation but they had different physiological status. Between the two groups of chicken dendritic cells, we found the final contents of the Newcastle disease virus had significant differences, and the chemokine (C-C motif) receptor 7, which can reflect the grade of maturation of chicken dendritic cells, ROBO2, interleukin 6 and interferon, gamma genes all had significantly differential expressions by real time PCR and Western blotting. These results suggested the mutation of ROBO2 gene could change the maturation of chicken dendritic cells to affect immune responses to Newcastle disease virus in chickens. Due to the serious damage of Newcastle disease virus, the ROBO2 gene can become an important gene in chicken breeding for disease resistance. Acknowledgments: This work was supported by the National Natural Science Foundation of China (Grant No. 31402067), the Pearl River Nova Program of Guangzhou (Grant No. 201506010017) and the Earmarked Fund for Modern Agro-Industry Technology Research System (Grant No. nycytx-42).

Keywords: ROBO2, dendritic cells, Newcastle disease virus, immune, maturation, chicken

S2-0139 Molecular mechanism of deposition of carotenoids on the chicken skin

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Carotenoids depositing in the skin epidermis makes yellow skin of animals. We extracted the yellow substance from the skin tissue of chicken, and determined it by high performance liquid chromatography. The result showed that there was no β -carotene at skin, but the deposition lutein and zeaxanthin caused the yellow skin in chicken. The beta carotene dioxygenase 2 which is encoded by BCO2 gene is a key enzyme in carotenoids oxidation decomposition process. Some studies indicated that the BCO2 has broad substrate specificity in carotenoid metabolism, and ferret BCO2 cleavage activity was higher toward zeaxanthin and lutein than β -cryptoxanthin in vitro. We found that the BCO2 gene expressed in the white skin, but not expressed in the yellow skin. On this basis, this project intended to discuss causes of the differential expression of BCO2 gene between white and yellow skins. Genetic variations in two different breeds of Guangxi yellow chicken (yellow skin) and Partridge shank chicken (white skin) were determined using the next-generation high throughput re-sequencing technology. The sequencing depth of each individual was $10 \times$ ($n = 5$), a total of $50 \times$ data each group. The results showed that Partridge shank chicken had a copy number variation (CNV) in the first intron of BCO2 gene (chr24:6147501- 6148300, 800bp). This CNV may be an important cause of the formation of white skin. Acknowledgments: This work was supported by the National Natural Science Foundation of China (Grant No. 31301968), the Science and Technology Program of Guangzhou (Grant No.201504010017) and the Earmarked Fund for Modern Agro-industry Technology Research System (Grant No. nycytx-42).

Keywords: chicken, BCO2 gene, copy number variations, yellow skin

S2- 0140 Expression and functional analysis of BMP7 in chicken

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Bone morphogenetic protein 7 (BMP7), a member of the BMP family is an attractive therapeutic agent for bone regeneration in humans and plays critical roles in skeletal development. We investigated the functional importance of BMP7 by analyzing its sequence and identifying polymorphisms associated with growth and body composition in different chicken populations. Here, a SNP (c.1995T>C) in BMP7 gene was identified, and the SNP was associated significantly with growth and carcass traits in the “Huiyang Bearded × Fast-growing Lingnanhuang Line A” F2 resource population. Furthermore, the body weights at 8-12 weeks of age, shank circumferences at 8 and 10 weeks of age, thigh bone and shank bone weights and metatarsal and phalanx weights for the birds with the TT genotype were significantly higher than those for the birds with the CC genotype ($P<0.05$). Genotyping revealed that the T allele occurred more frequently in breeds with high growth rates, whereas the C allele was predominant in those with low growth rates. The expression level of BMP7 in the thigh bone of birds with the TT genotype was significantly higher than in those with the CC genotype at 21, 42 and 91 days of age. This suggests that the T allele is favorable for improving body weight and bone growth in broilers. Our results provide evidence that the SNP c.1995T>C could serve as a selective marker for improving bone growth and increasing the consistency of body weights in poultry breeding. Acknowledgments: This study was supported by the Operating Fund for Laboratory System Construction and Opening of Guangdong Province, the Operating Funds for Guangdong Provincial Key Laboratory of Animal Breeding and Nutrition (Grant No. 2014B030301054) and the Ear-marked Fund for Modern Agro-Industry Technology Research System (Grant No. nycytx-42).

Keywords: bone formation, shank circumference, body weight

S2-0141 Nonsense mutation in TVB gene result in resistance to subgroup B avian leukosis virus

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Different genetic variations in receptor for avian leucosis virus (ALV) can cause hosts resistance or susceptibility to ALV. Receptor for subgroup B avian leucosis virus (ALV-B) is encoded by tvb gene. A new single nucleotide polymorphism (SNP) at position 3674 (C>T) of tvb genomic DNA, named tvbr3, was identified in Chinese native chicken breeds. In this study, decreased susceptibility to ALV-B was observed in tvbr3/r3Chicken embryo fibroblasts (CEFs). DNA sequence analysis showed that the resistance to infection by ALV-B may be explained by a premature stop codon caused by the natural mutation of 3673(C>T). To better understand the mechanism of resistance to viral infection induced by mutations in tvb, we constructed expression plasmid pET32a- CC/TT encoding wild tvb gene (tvbs) and tvbr3 separately, expressed by prokaryotic expression system. An approximately 20kDa protein was detected by Western blot, which is smaller than wild tvb protein. It confirmed that this nonsense mutation in tvbr3 caused the expression of TVB protein prematurely terminated, which could affect the binding affinity of receptor and decreased the virus entry.

Keywords: tvbr3, ALV-B, nonsense mutation

S2-0142 Goose TGH gene negatively regulate formation of fatty liver

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It has been reported that TGH gene played important roles in mobilization of intracellular TG stores for VLDL secretion. To study the molecular mechanism of TGH gene functioning in goose fatty liver development, the Landes goose TGH gene was re-sequenced and its transcripts were also cloned using RACE method. The expression profiles of TGH gene during different periods in overfeeding experiments were also investigated. The results showed that the goose TGH gene code two transcripts, a longer X1 transcript encoding 575 aa and a shorter X2 transcript encoding 557 aa. Both the amino acid sequences have high similarities with those of duck. TGH gene specifically expressed in liver and had highest mRNA level in liver but nearly no expression in skin, brain, adipose or heart. The expression levels of TGH gene down-regulated when the liver size enlarged along with the overfeeding ($P < 0.01$). However, the expression level kept a high level in control groups and it also increased to a high level when overfed stopped ($P < 0.01$). However, there was no genetic variations found in the UTRs, introns or exons of goose TGH gene which might indicated high conservation in Landes breed. In conclusion, its negative correlations of TGH gene expression levels with livers or hepatocytes size functioned in the lipids deposition during the development of fatty liver.

Keywords: goose, TGH gene, cloning, expression, fatty liver

S2- 0143 A comparison of genomic prediction accuracy between feed conversion ratio and residual feed intake in meat-type chickens

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Feed represents the major cost of chicken production. Selection on feed utilization traits is a feasible way to reduce feed cost and greenhouse gases emission. The objective of this study was to investigate the efficiency of genomic prediction for feed conversion ratio (FCR) and residual feed intake (RFI) in meat-type chickens. The data included 504 birds from 8 half-sib family. The birds were genotyped using the Illumina Chicken 60K SNP Beadchip. Genomic prediction was assessed using a 4-fold cross-validation for two validation scenarios. The first scenario is random family sampling (CVF) and the second scenario is random individual sampling (CVR). Genetic variances were estimated based on single nucleotide polymorphism markers. Genomic estimated breeding values were predicted using a genomic best linear unbiased prediction model. The heritability estimates of FCR and RFI were 0.29 and 0.50, respectively. In both scenarios, the accuracy of GEBV for FCR was lower than RFI. The accuracies of predictions for FCR, RFI, average daily gain (ADG) and average daily feed intake (ADFI) were 0.194, 0.200, 0.401 and 0.378 in CVF scenario, and 0.241, 0.417, 0.406 and 0.455 in CVR scenario, respectively. These results indicate that RFI and FCR have moderately genomic prediction accuracy. Compared with FCR which can be improved by selection for ADG, selection for RFI could be a good alternative to selection for improving feed efficiency in meat-type chicken breeding programs.

Keywords: genomic selection, feed efficiency, feed conversion ratio, residual feed intake

S2-0145 Identification of differentially expressed proteins in preadipocytes of broiler chickens divergently selected for abdominal fat content

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Decreasing excessive body fat deposition is one of the main goals for the poultry breeding. Preadipocytes play an important role in adipose tissue growth and development. The objective of present study was to identify the differentially expressed proteins in preadipocytes of fat and lean broiler lines. The chicken preadipocytes in this study were derived from Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHLF). The differentially expressed proteins were separated by two-dimensional difference gel electrophoresis (2-D DIGE) approached and identified by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS). A total of 46 differentially expressed protein spots were found in the preadipocytes between the fat and lean broilers. After MALDI-TOF-MS analysis, these protein spots corresponding to 33 different proteins were identified. These proteins were divided into seven categories according to their functions, such as biological oxidation, cytoskeleton, lipid metabolism, molecular chaperone protein synthesis, and signal transduction. Among the identified proteins, NDUFS3 (NADH dehydrogenase [ubiquinone] iron-sulfur protein 3), NDUFV2 (NADH dehydrogenase 24 kDa subunit), QCR1 (cytochrome b-c1 complex subunit 1, mitochondrial), SAMM50 (sorting and assembly machinery component 50 homolog A), lamin-A and Napb protein are interesting candidates for further study on chicken fat deposition.

Keywords: Fat deposition, broiler, preadipocyte, differentially expressed protein, 2-D DIGE

S2-0147 Computer tomograph study for testing the usability of a newly developed cock line for improving the meat production of the TETRA-H dual-purpose chicken genotype

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The aim of this study was testing the effectiveness of the use of a newly developed cock line in the breeding program of the TETRA-H dual-purpose chicken genotype for improving its meat production. For this purpose changes in the body composition of TETRA-H chickens, chickens of a newly developed cock line (originated from Golden Plymouth and selected for weight gain and plumage color) and chickens of the improved TETRA-H (TETRA HB Color) were compared during the rearing period. The experiment was carried out with altogether 90 chickens (15 in both sexes in each genotype), which were scanned bi-weekly by means of computer tomography (CT) between 4 and 10 weeks of age. During the scanning procedure a total of 20 scans (cross-sectional CT images) were taken from each animal, using 8 mm slice thickness and different distances between the scans, depending on the length of the vertebrae. From the images obtained three-dimensional histograms were created by the negative exponential interpolation method using the pixels with X-ray density values of muscle, water and fat, i.e. the range between -200 to +200 on the Hounsfield-scale. Using these histograms for demonstrating changes in the body composition of the chickens it was established that the use of the newly developed cock line resulted in an improved meat production in the TETRA HB Color chickens, mainly in the amount of the breast muscle. The volume of the breast muscle of the TETRA HB Color chickens was between the volume of the TETRA-H chickens and chickens of the newly developed cock line at all examination days, but it was always closer to that of the TETRA-H chickens. However, the use of the newly developed cock line resulted not only in the improvement of the meat production of the TETRA HB Color chickens, but also in an increase in their amount of abdominal fat, especially in the case of the pullets in the last two weeks of the experiment.

Keywords: chicken, dual-purpose, body composition, meat production, computer tomography

S2- 0148 The comparison of blood characteristics in low and high altitude chickens

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Tibetan chicken (*Gallus gallus*) is a specific chicken breed inhabiting the Tibet Plateau for thousands of years, and has thus adapted to the harsh environment at the altitude of over 4000 m, especially the chronic hypoxia and low pressure. The changes in blood characteristics that take place during high altitude rearing of Tibetan chickens are unknown. In order to get a deep insight of blood characteristics in low and high altitude chickens, we focused on four chicken breeds to compare the variations among them. In the present study, we found significant differences in WBC (white blood cell count), RBC (red blood cell count) and HCT (hematocrit) values between Tibetan chicken and other chicken breeds. When Tibetan chickens were reared at low altitude, there was a significant ($p < 0.05$) decrease in WBC, RBC and HCT values compared to the Tibetan chickens (TC) reared at high altitude, but whether the mean of MCV (mean cell volume) value is related to oxygen transport and hypoxia adaptation requires further study. It is likely that MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration) and PLT (platelet count) values are lowered down at high altitude hypoxic environment. So the increase in WBC, RBC and HCT may be the common hematological mechanism for Tibetan chickens to adapt to high altitude hypoxia. These research results provide the theory of scientific basis for Tibetan chickens to adapt to high altitude hypoxia.

Keywords: hypoxia, Tibetan chicken, high-altitude adaptation, blood characteristics

S2- 0149 Tendency to lay eggs on floor is hereditary

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Floor eggs from laying hens kept in floor/aviary or free range systems is not acceptable due to the labor in picking the eggs and poor hygienic standard of these eggs. The reason for the increase in numbers of hens kept in that way is the ban of traditional cages for laying hen from 2012 in the EEC countries and the movements of consumers, food stores and fast food restaurants in USA away from eggs laid by hens in cages. This means that a considerable part of the future laying hens is going to be kept in a floor/aviary system or in free range. Keeping hens in multitier systems, including floor/free range has given problems with too many interactions between hens resulting in cannibalism. Worldwide breeding companies have breeding stocks, been bred for high yield through many generations based on the individual yield when measured in cages. These hens have improved considerably in egg yield, but may have lost some of their ability to interact with pen mates when kept on floor or free range in larger groups; they may also have lost some of their willingness to visit the nest for laying an egg. There seems to be developed breeding system that would be able to reduce unacceptable strong interaction among hens and still keep the hens for test and selection in a modified cage system. Breeding for less floor eggs will not be advanced in such a system. During a selection experiment with a base population of 7 international hybrids of laying type hen crossed together, we proved that the proportion of floor eggs were reduced with 9% per generation or 45% over the 5 generations, compared to the control line, when measuring the egg yield in a floor system with a trap nesting system. The 7 international hybrids were expected to be selected for high egg yield based on individual cages. It is believed that this change in frequency of floor eggs has a hereditary back ground.

Keywords: floor eggs, hereditary, management system, breeding

S2- 0150 Eggshell and bone quality in two different genetic groups of aged layer breeders

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The present study was conducted on two aged commercial laying breeder strains (Hy-Line Brown and Hy-Line W-36) to evaluate genetic differences of eggshell and bone quality (at 71 and 72 wks of breeders' age, respectively). In addition, mortality rate, egg production performance (Beginning from 60 to 70 weeks of layer breeders' age), internal organs and reproductive measurements (at 72 wks of breeders' age) were also determined. Brown layer breeder strain at the end of laying cycle had significantly higher mortality rate compared to the white one. White layer breeder hens had significantly higher percentages of hen-day and hen-housed egg production (59.91% and 45.82%, respectively) compared to the brown one (55.27% and 35.27%, respectively). Brown Hy-Line layer breeder strain had better eggshell quality (wet or dry shell weight, shell percentage, shell thickness and eggshell breaking strength) than W-36 Hy-Line layer breeder strain. While, no significant difference was detected between the two layer breeder strains for egg weight or egg shape index. No significant differences between breeder strains for shank length, relative liver weight and abdominal fat percentage were detected. The white layer breeder strain had significantly higher relative oviduct weight (4.54%) compared to the brown one (3.23%). Brown layer breeder strain recorded higher humerus width (8.25mm) in comparison with the white one (7.90mm). Tibia bone weight of brown layer breeder strain had significantly heavier by 22.87% as compared to the white strain. Also, the brown breeder strain recorded higher tibia width, thickness and strength in comparison with the white one. Tibia bone strength of brown layer breeder strain had greater by 33% than that of the W-36 layer breeder strain. A positive correlation coefficients were detected between humerus bone strength and both eggshell thickness and strength in brown and white strains.

Keywords: eggshell, bone quality, breeders strain

S2- 0151 Identification of TGFBR2 isoforms in chicken

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Several isoforms of transforming growth factor beta receptor II (TGFBR2) have been identified in mammals. However, no TGFBR2 isoforms have been reported in chickens. The objective of the current study was to identify chicken TGFBR2 isoforms. Total RNA was isolated from different chicken tissues and reverse transcribed to cDNA. PCR was performed to amplify the full-length coding region of chicken TGFBR2 with the following specific primers: forward, TCTCCGGCTCCGCGATGC; reverse, TAGAAGCGACCTCCCTCC. Agarose gel electrophoresis analysis showed the PCR amplification generated two different sized PCR products: approximately 1.8 kb and 2.1 kb. These two PCR products were purified, cloned, sequenced. Sequence analysis by DNAMAN and blast software showed that the sequence of the small PCR product was identical to the known chicken TGFBR2 mRNA sequence, which is designated as isoform 1. The sequence of the larger PCR product was almost identical to that of isoform1 except for an insertion of 342 bp, which is designated as isoform 2. ORF analysis showed that isoform 1 encodes a protein of 557 amino acids, but isoform 2 encodes a protein of 671 amino acids. BLAT analysis showed that both isoforms were generated from chicken TGFBR2 gene by different alternative splicing events. Isoform 1 contains 7 exons, and isoform 2 comprises 9 exons. Further analysis showed that the isoform 2 has a repeat of exons 2 and 3, and this repeat is due to a 3- kb genomic duplication within TGFBR2 gene, which contains exons 2 and 3. Expression analysis showed that both two TGFBR2 isoforms were expressed in the tested 12 tissues, but comparatively, the isoform 2 was highly expressed in most of the tested tissues. In conclusion, two chicken TGFBR2 isoforms were identified.

Keywords: chicken, TGFBR2, alternative splicing, isoform

S2-0152 Immortalization of chicken preadipocytes by retrovirally transduced chicken TERT and TR

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Our goal was to establish an immortalized chicken preadipocyte cell line by chicken telomerase activity restoration. Two core components of chicken telomerase, chTERT and chTR, were cloned, inserted into retroviral expression vector, and packaged into viral particles. primary chicken preadipocytes were infected with the recombinant retroviruses, selected and characterized. The results showed that two immortalized chicken preadipocyte cell lines were successfully generated. Both the chicken preadipocytes have survived over 100 population doublings. TRAP assay showed these two cell lines gained high telomerase activity, and β -gal staining displayed that they had no signs of replicative senescence. Similar to primary chicken preadipocytes, the two cell lines displayed a fibroblast-like shape, and retained the capability to differentiate to adipocytes, as demonstrated by Oil Red O staining. These two immortalized chicken preadipocyte lines show great promise as in vitro models for the investigation of chicken adipogenesis and its related diseases, and our results also provide clues for immortalizing other avian cell types.

Keywords: preadipocyte, telomerase, immortalization, chicken

S2-0153 The reanalysis of white feather gene by the genome-wide association study

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Introduction Chicken (*Gallus gallus domestica*) plumage is regulated by multiple genes. Chicken feather color is related to pigment distribution, content and ratio. Chicken feather color common traits and gene mapping information has been progress in certain studies. As we all known, Dominant white feather is regulated by PMEL17 gene and Recessive white feather is regulated by TYR gene. **Materials and methods** We constructed an F2 resource population by crossing of the White Leghorn chicken and Recessive White Plymouth chicken, and recorded feather color of the F2 chickens at birth and adult stage. Then we genotyped the F2 chicken by using the chicken 600K SNP chip from Affymetrix company. We also genotyped the known PMEL17 gene and TYR gene mutations associated with dominant or recessive white. Genome-wide association study was Carried out with the software Plink1.9 and visualized the associated results with R. **Results and discussion** According to chicks phenotype, we found a significant SNP site, which was located in Linkage Group: LGE22C19W28_E50C23. Through adult chicken feather color, it associated with chromosome 1 zone, and linkaged with TYR gene. In this study ,interestingly, Chicken plumage phenotypic changed at different growth stages .It may be the Dominant white gene PMEL17 in the chick stage, and may be Recessive white gene TYR in the adult stage.The reason for the result may be the phenotype varied in different life stages so that we cannot get the accurate phenotype.In addition, when we genotyped PMEL17 and TYR gene,we found some white feather chickens with genotype CCii or Ccii,so we thought it might have new white gene.After the correction of PMEL17 and TYR gene , we found no significant SNP loci on the genomic level ,but there was a significant SNP site the chromosome 9 , indicating that there may be have new white gene or other mechanism. But the effect was small, it needs further analysis and experiment verification.

Keywords: white feather gene, PMEL17, TYR, genome-wide association study

S2-0154 Response to three generation of divergent selection for shank length in Japanese quail

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Three quail lines were used in the present experiment to study the effect of divergent selection for shank length (SL) for three generation after established the base population, the LSL28 line, established for selection for long SL, the SSL28 line, short SL at 28 days of age and random bred control line SL and body weight (BW) were recorded at hatch, seven, 14, 21, 28, 35 days of age. Age at first egg (AFE), egg weight (EW) of the 1st, 10th, 30th, 60th and 70th egg, number of days needed (DN) to produce the first 10, 30, 60 and 70 eggs and egg mass (EM) of the first 10, 30, 60 and 70 eggs and hatching traits were recorded for each female. The G2 had remarkably longer SL at one, 14, 21, 35 days of age and G3 had longer SL7 than other generations. The G2 had higher BW_{hatch} and BW₁₄ than other generations, the G3 had heavier BW₂₁, BW₂₈ and BW₃₅. The LSL28 had longer SL's at different ages studied from day-old up to 35 days of age. The LSL28 line had significantly heavier BW_{hatch}, seven, 14, 21 and 28 whereas quail of the SSL28 line had the lightest BW_{hatch} and BW₁₄. The G1 of selection had significantly heavier EW_{First}, EW₁₀, EW₃₀, EW₆₀ and EW₇₀, EM₁₀, EM₃₀, EM₆₀ and EM₇₀ than other generations but needed undesirable longer DN₃₀, DN₆₀ and DN₇₀. The G3 had higher, desirable lower DN to produce the first 30, 60 and 70eggs but, lighter EW_{First} and latest AFE. The G2 attained sexual maturity at earlier age than other generations. All hatching traits studied were significantly influenced by generation effect. The LSL28 line had significant shorter DN₁₀, DN₃₀, DN₆₀, DN₇₀, heavier EM₁₀, EM₃₀, EM₆₀, EM₇₀, earlier AFE, heavier EW_{First}, EW₁₀, EW₃₀, EW₆₀, EW₇₀ than other lines. Line insignificantly affected all hatching traits studied. In conclusion, selection for SL28 had significant desirable correlated responses were evident for body weight and most of egg productio and hatching traits.

Keywords: quail, selection, shank length, productive performance.

S2-0155 MicroRNA sequencing data to identify miRNAs affecting Kirin chicken follicle development

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As the derivative of chicken skin, hair follicle is capable of self-renew. Its proliferation and differentiation result in hair formation. MicroRNAs (miRNAs) can effectively regulate gene expression at the post-transcriptional level and play a critical role in tissue growth, development. In this study, we used next generation sequencing technology sequenced miRNAs of the hair follicle derived from the 13 day-old chicken (*Gallus gallus*) embryos in which from Kirin chicken and Huaixiang chicken that feathers having morphogenesis with significantly different curling. A population of conserved miRNAs was identified. These conserved miRNAs were derived from 638 homologous hairpin precursors across 5 animal species. We identified a total of 645 miRNAs in the chicken embryos. Among them, 10 differentially expressed miRNAs were identified ($\geq \pm 2$ Fold, p value < 0.05) by comparing Kirin chicken and Huaixiang chicken. Several gene ontology (GO) biology processes and the WNT, BMP and TGF- β signaling pathways were found to be differentially expressed miRNAs as part of hair follicle development process. This study has identified miRNAs that associated with the chick embryonic hair follicle development and identified some target miRNAs for further research into their role played in feather growth.

Keywords: Kirin chicken, follicle, miRNAs

S2-0156 Expression of long noncoding RNAs in the bursa of fabricius and melanocytes of Silky Fowl

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To investigate the melanocytes' function in Silky Fowl (SF) can help us to rational utilization of this genetic resource. Our previous studies have found that lower expressions of immune genes were in the SF embryo, and aberrant immune system development was detected during the early development stage. In order to illustrate the melanocytes' function on B cells development in bursa of fabricius (BF), 3 female SFs and 3 female White Leghorns (WLs), aged at 3 weeks old were obtained. Compared with WL, the index of BF and B cells in SF were significantly lower ($P < 0.05$). Recently, studies showed us that long noncoding RNA (lncRNA) had important roles in the regulation of immune cells development. So the lncRNA expressions in the BFs (lncBF) were explored by RNA-seq to discover special lncRNAs in melanocytes, which might modulate B cells development. There are 326 differentially expressed lncBFs in BF of SF with 258 over-expression and 68 low-expression. We chose and quantified 19 highly and 3 lowly expressed lncBFs in the BF by qPCR. In addition, 3 highly and 3 lowly expressed lncBFs were confirmed in the cultured melanocytes in vitro. It is reported that noncoding RNA in vesicle could be secreted from keratinocytes, and be adopted by melanocytes to modulate the melanin synthesis. So we speculated that the lncBFs in melanocytes might have the role in modulating B cells development. Using RNAfold and RNAs-structure software, cervical-loop structures were predicted in the lncBFs, which would be beneficial to fulfill their work after interaction with special proteins. The precise mechanism of lncBFs' regulation on B cells development needs further to be elucidated.

Keywords: Silky Fowl, long noncoding RNA, B cell, melanocyte, development

S2-0157 Two novel variants of chicken GPR133 and their expression in different tissues

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GPR133 (termed ADGRD1: adhesion G protein-coupled receptor D1 in chicken) is a member of the Adhesion GPCR family. The family are coded for by many exons and their genomic structure is in general complex, which makes them a challenging group to study. GPR133 plays an important role in various tissues, two functional alternative splicing (AS) were found for GPR133 in human, while little information is available about the AS of chicken GPR133(cGPR133) mRNA. In the present study, we identified two variants in chicken by a combination of RT-PCR and rapid amplification of cDNA 5'-ends (5' RACE). Sequence analysis showed that two mRNA sequences of cGPR133 were obtained: cGPR133-va (3289 bp) and cGPR133-vb (3385 bp). Searching the ensembl database identified cGPR133 gene, which contained a 138.53 kb sequence from chromosome 15 and consists of 26 exons and 25 introns. The nucleotide sequences of exons were found to be completely retain to cGPR133-vb. However, cGPR133-va only contains 25 exons and a exon is missing. The expression of cGPR133-va and cGPR133-vb was examined in 31 chicken tissues using qRT-PCR. Quantitative real-time PCR (qRT-PCR) analysis revealed that all two variants were widely expressed in chicken tissues, and predominant expression of all two variants were respectively detected in the eye, pituitary gland, oviduct, heart, muscular stomach and lung tissues. More interestingly, high level expression of cGPR133-va was found in specific fat tissues (sebum and abdominal fat), while cGPR133-vb was detected in hierarchical follicle (P:F1-F5). We speculated that cGPR133-va may play a critical role in fat tissues, while cGPR133-vb may play a critical role in hierarchical follicle. Thus, we provide the first AS of the cGPR133 and implied that the two cGPR133 variants were functional in chicken organisms.

Keywords: chicken, GPR133, Alternative splicing, expression, different tissues

S2-0158 Effects of strains and ambient temperature and their interaction on production performance, egg quality and physiological response of laying hens

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The present experiment was undertaken to compare the performance, egg quality, blood properties and stress responses between the Bangladesh Livestock Research Institute (BLRI) developed layer strains and commercial layer strain under thermo-neutral and heat stress condition of Bangladesh. A total of 192 ready to lay pullets were randomly assigned to a 3 × 2 factorial arrangement of treatments (4 replicate/treatment; 8 birds/replication) consisting of three layer strain (Shuvra, Shorna and Hyline white commercial layer strain) and two ambient temperatures (heat stress 30-32°C; thermo-neutral 18-20°C). Results showed that body weight was significantly ($P<0.05$) increased in Shorna than that of Shuvra and commercial hens. The interaction between strain and temperature were not significantly influence the rate of egg production. The effect of strain on egg weight was significant ($P<0.01$) and thus increased egg mass production by the Shorna than that of commercial strain. With increasing environmental temperature from thermo neutral condition to heat stress condition plasma glucose level increased to the significant level. A higher number of follicles were found in the shorna strain than that of other two strains ($P<0.05$). When temperature changed from 20°C to 32°C, ovarian follicle number was significantly reduced ($P<0.05$) and this effect is more apparent to the commercial hen. Bird's warm carcass weight and muscular pH were obtained higher in Shorna than that of Shuvra and commercial strain. But pH level of breast muscle was notably declined in heat stressed bird than that of thermo-neutral groups. Thus, the present results indicated that Shuvra and Shorna are comparable with the commercial strain, suggesting physiologically adaptable under existing environmental condition of Bangladesh.

Keywords: strain temperature, performance, egg quality, physiology and laying hens

S2- 0159 shRNA- Decorin inhibits the proliferation of duck (Anas platyrhynchos) myoblasts by IGF-1/PI3K/AKT/mTOR and TGF-β/Smad pathway

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Decorin (DCN), presenting in the extracellular matrix, is a member of the chondroitin sulfate proteoglycan gene family. DCN is an extracellular ligand of TGF-beta pathway, and affects the differentiation and metastasis of myoblast through this signaling pathway. DCN promotes cell proliferation and differentiation by inhibiting MSTN. A higher expression of IGF-1R was detected in DCN insufficient endothelial cells. DCN gene can promote C2C12 myoblast differentiation through activating AKT. In this study, we constructed plasmid vector (shRNA3) to silence the function of DCN. The expression of DCN was suppressed in duck myoblasts and leg muscle by shRNA3, and the proliferation of myoblast was inhibited. The mRNA level of IGF-1R, PI3K, AKT, MTOR, P70S6K, MYOD, TGF-βR1, and Smad4 genes and DCN, PI3K, MTOR, TGF-βR1, and Smad2 proteins were significantly reduced in myoblasts transfected with shRNA3 ($P<0.05$) compared to that transfected with shControl. Subsequently, compared with the myoblasts transfected with shRNA3 without RH IGF-1, cell proliferation activity was enhanced, and the mRNA level of IGF-1R, AKT, MTOR, P70S6K, and Smad4 genes and DCN, PI3K, MTOR, TGF-βR1, and Smad2 proteins were significantly increased in myoblasts transfected with shRNA3 in the presence of RH IGF-1. Silencing DCN by shRNA3 inhibited the mRNA level of IGF-1R, MTOR, S6K, MSTN, TGFβR1, and Smad4 genes in duck leg muscle. But the DCN, MTOR, and Smad2 proteins level were significantly increased, and there had no significantly impact on muscle morphology. These results suggested that shRNA-Decorin inhibits the proliferation of duck (Anas platyrhynchos) myoblasts by IGF-1/PI3K/AKT/mTOR signaling and TGF-β/Smad signaling pathway, but its transitory expression had no significant effect on duck leg muscle.

Keywords: decorin, duck, myoblast

S2-0161 Analysis of major gene and polygene mixed inheritance model for chicken laying rate in different weeks

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White Lenghon and Bule-eggshell Chicken as P1 and P2 were selected in this study, F1 and F2 were derived from reciprocal crosses P1 and P2. The data set contained records of laying rate rate from age at first egg to 40 weeks, 72 weeks and 40-72 weeks, respectively, the genetic parameters were estimated by applying the major gene plus poly-gene model of quantitative traits of SEA-G4F2. It is recommended that the optimum model for 40 weeks is E-1, and the model E are best for 72 weeks and 40-72weeks. The E-1 and E model, namely two pairs of additive-dominance-epistatic major-gene plus additive-dominance polygene mixed genetic model and two pairs of additive-dominance-epistatic major-gene plus additive-dominance-epistatic polygene mixed genetic model. The heritability of major-gene were 35.43%, 37.17% and 41.96% respectively, while the polygene were 0. The current study suggested that selection in laying rate will be an effective way in breeding.

Keywords: laying rate, major gene, genetic model

S2-0163 The effect of transcription regulation activity of G-198A Mutation in 5' flanking region of chicken CAST gene

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The calpain proteinases and their specific inhibitor calpastatin have been proposed to influence both the rates of myofibrillar protein turnover in vivo and meat tenderization postmortem. The aim of this study was to analyze the promoter activity of different genotypes of G-198A mutation in 5' flanking region of CAST gene were detected by Dual-Luciferase Reporter Assay. The results will provide a reliable basis for marker-assisted selection for breeding of high quality broiler and expression and function of CAST gene. The CAST517-promoter of CAST gene was amplified using PCR technology, inserted into the dual-luciferase reporter vector (pGL3-Basic) to construct pGL3-CAST517/promoter. 293T cell were cotransfected with a plasmid containing the pGL3-CAST517/promoter and pGL3-Basic, respectively. The expression of luciferase gene in 293T cell was tested using the Dual-Luciferase Reporter Assay kit. Binding sites and transcription factors of CAST517 sequence were predicted by online tools and analysis the transcription level of different genotypes. The promoter effects of CAST517A/G were tested using dual reporter gene assay showed that the ratio of renilla to firefly fluorescence intensity of CAST517A increased 113.89% ($P < 0.01$) than that of CAST517G, which indicated that the promoter efficiency was improved when A replaced with G at G-198A site. Computational analysis of CAST517 sequence of CAST gene were performed using the BDGP and TFSEARCH database and one TATA box, one transcription starting point, three potential cis regulatory elements (PR, ER and GATA-1) and one potential trans regulation (TGGCA-binding) were found. These results implied that individuals with CAST517A have higher transcription level and can improve the quality of chicken meat. The results provide a theoretical basis for the breeding of broiler chickens.

Keywords: broiler, calpastatin, promoter, meat quality traits, luciferase reporter gene

S2- 0164 Estimation of the effective size of population for three high quality native chickens in Jiangxi Province of China

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The method based on linkage disequilibrium (LD) has advantages over other methods. In the present study, 30 SNPs (Single Nucleotide Polymorphisms) located in a 500 Kb region within a Contig.060226.1 on GGA1 were selected to study the SNP diversity, linkage disequilibrium, and calculation of the effective population size (Ne) for three high quality native chicken (Ningdu Huang chicken, Baier Huang chicken and Anyi Wuhui Chicken) in Jiangxi province of China. Our data indicated that Ne is greater at the early stages of domestication ($t=769$), Ne of Ningdu Huang chicken, Baier Huang chicken and Anyi Wuhui chicken are 4074, 3090 and 1480, respectively. At the later stages of domestication, Ne of Ningdu Huang chicken is 550 ($t \leq 59$), and Ne of Baier Huang chicken and Anyi Wuhui chicken are 300 and 277, respectively ($t \leq 45$). The Sved formula was fitted to the LD value (r^2) and the physical distance. We found that the regressions are significant for Ningdu Huang chicken ($P=0.0272$, $R^2=0.9196$), and Anyi Wuhui chicken ($P=0.0193$, $R^2=0.7276$). Although the regression is not significant for Baier Huang chicken ($P=0.3837$, $R^2=0.2541$), 25.41% of the total variation of r^2 could be explained by the transmitted physical distance. We argue that the estimation of Ne for Ningdu Huang chicken and Baier Huang chicken are accurate relatively, the estimation for Baier Huang chicken is more likely to be bias. This study has important theoretical significance in situ preservation for three high quality native chickens, especially for Ningdu Huang chicken and Anyi Wuhui chicken.

Keywords: Ningdu Huang chicken, Baier Huang chicken, Anyi Wuhui chicken, linkage disequilibrium, effective population size

S2- 0165 Long intergenic non- coding RNA GALMD3 associates with chicken Marek's disease

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Long intergenic non- coding RNAs (lincRNAs; > 200 nucleotides) are transcribed from non- coding DNA sequences between coding genes. So far, thousands of lincRNAs have been cataloged in human, mouse, zebrafish, and other species. Studies have revealed that aberrant expression of lincRNAs is associated with various types of cancers and neurological disorder. Marek's disease (MD) is a highly contagious T- cell lymphoid neoplasia of chicken induced by Marek's disease virus (MDV), which causes huge economic losses to the poultry industry. In this study, we discovered 70 lincRNAs in MDV-induced chicken CD4+T cells isolated from two reciprocal cross lines, which were line 63 (MD resistant) \times line 72 (MD susceptible), and line 72 \times line 63. Linc-GALMD3 was first identified and validated highly expressed in MDV-infected CD4+T cells in both two reciprocal cross lines. MDCC-MSB1, as an MDV-transformed CD4+T cell line, was used to explore the biological significance of linc-GALMD3. The short hairpin RNAs (shRNAs) were used to knockdown the expression of linc-GALMD3 in MDCC-MSB1 cells, and the highest interference efficiency was 53% (shRNA3-1657). The RNA-sequencing was performed before and after linc- GALMD3 knockdown by shRNA3-1657, and 748 differentially expressed genes (DEGs) were found ($FDR < 0.01$). These DEGs were significantly clustered in cell cycle, mitotic cell cycle, cell cycle process, and cell cycle phase after GO enrichment. Our findings provided data resources for studies on lincRNAs in MD, moreover, linc-GALMD3 was first discovered and might play a critical role in MDV tumorigenesis.

Keywords: chicken, Marek' s disease, linc-GALMD3, shRNAs, RNA-Seq

S2- 0166 gga-miR-99a plays a key role in *Mycoplasma gallisepticum* (HS Strain) infection of chicken

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Mycoplasma gallisepticum (MG), one of the most pathogenic *Mycoplasma*, has caused tremendous economic loss in the poultry industry. Recently, increasing evidence has suggested that micro ribonucleic acids (miRNAs) are involved in microbial pathogenesis. However, little is known about potential roles of miRNAs in chicken's MG infection. In the present study, we have found that gga-miR-99a was down-regulated in the lungs of MG-infected chicken embryos by using miRNAs solexa sequencing. The expression of gga-miR-99a in the tissues and DF1 cells which infected or uninfected by MG-HS is tested by Q-PCR. Target genes of gga-miR-99a was predicted by bioinformatics analysis and proved by dual-luciferase reporter assay. The expression level of gga-miR-99a was up-regulated and down-regulated to find the relationship between gga-miR-99a and the target gene. The cell proliferation rate of DF1 cells which transfected by gga-miR-99a mimics was tested by MTT, and the cell cycle was tested by flow cytometry. The main results are as follows: (1) The expression of gga-miR-99a in the tissues of MG-HS infected lung is extremely significant increased ($P < 0.01$) at E13, E14, E16, E17 while extremely significant decreased ($P < 0.01$) at E12, E15, E19, E20. Moreover, the expression of gga-miR-99a in MG infected DF1 cells was higher than normal cells ($P < 0.01$). (2) The result of dual luciferase reporter assay and Q-PCR show that over-expression of gga-miR-99a significantly inhibits SMARCA5 expression ($P < 0.01$). (3) The result of Q-PCR shows the relative expression trend of SMARCA5 in the infected lung tissue of chicken embryos was opposite to gga-miR-99a at E12, E16. The expression of SMARCA5 in DF-1 cells infected by MG-HS were decreased significantly ($P < 0.01$). (4) gga-miR-99a can prevent DF1 cells entering S phase from G1 phase, and suppress the proliferation of DF-1 cells.

Keywords: Chicken; *Mycoplasma gallisepticum* (HS strain); gga-miR-99a; SMARCA5

S2- 0167 Influence of lighting programme on amino acid requirements in a broiler breeders flock

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This study modelled the changes in body weight (BW) and composition (BC) in broiler breeder pullets up to the point when all the birds in the flock had laid their first egg, in response to two lighting programmes during the rearing period. The growth of each individual in a population from hatching to the time when its first egg is laid was simulated and then averaged to obtain the mean response of the population to two lighting programmes. The information about the age, BW and BC over the growth period is required in order to determine the amino acid requirements of broiler breeder hens, the age at first egg (AFE) and egg production (EP). The amino acids requirement was determined according to the lighting programme used during the rearing phase. Were used equations to estimate body, feather, ovary, oviduct and compositions of these parts, as a function of age. Also was estimated AFE and EP according to the lighting programmes (A: 8h light/d to 100 d followed by an increase at that age to 13h/d; B: 8h light/d to 140 d at which age the light was increased to 13 h/d). It is important to point out the greater requirements for the lighting programme A compared to B, and this occurs until all the broiler breeders begin laying egg. The requirements of Lys were 571 ± 303 and 466 ± 258 and Met+Cys were 501 ± 303 and 399 ± 258 at 24 week of age. For 28 weeks of age, the requirements of Lys were 718 ± 352 and 927 ± 265 and Met+Cys were 635 ± 352 and 832 ± 265 . For 32 weeks of age the requirements of Lys were 910 ± 336 and 957 ± 308 and Met+Cys were 812 ± 336 and 855 ± 308 mg/d, for lighting programme A and B, respectively. Knowing the requirements helps to establish the best nutritional program, and optimize feeding without excesses and deficiencies. These results support the view that a modelling approach has the potential to help nutritionists to make decisions about feeding broiler breeder flocks more efficiently.

Keywords: amino acids requirement, simulation, stochastic model

S2-0168 Modelling the egg components in laying hens

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This study aimed to model the egg components of laying hens during the egg production period. One hundred and twenty Hy-Line W36 and ISA Brown layers were used from 18 to 60 weeks of age with each bird being an experimental unit. The birds were housed in individual cages during the experimental period. Egg weight (g) and the weight of yolk, albumen and shell were recorded for each bird. The data were used to estimate the parameters of the equations for predicting the weights of yolk, albumen and shell. The result of egg weight were evaluated by regressing residual (observed minus predicted) values on the predicted values centred on their mean value. The equations for predicting mean yolk weight with age are $y_1 = 13.6 \times (1 - e^{-0.0207 \times (\text{Age} - 81.46)}) \times e^{0.00053 \times \text{Age}}$ and $y_2 = 15.3 \times (1 - e^{-0.0207 \times (\text{Age} - 109.4)}) \times e^{0.00011 \times \text{Age}}$ for Hy-Line W36 (y_1) and ISA-Brown (y_2), respectively. Albumen and shell weights for Hy-Line W36 were described by the equations $15.07 \times (\text{yolk weight})^{0.37}$ and $0.70 \times (\text{yolk} + \text{albumen weight})^{0.50}$, respectively, and for ISA-Brown, $21.99 \times (\text{yolk weight})^{0.24}$ and $1.60 \times (\text{yolk} + \text{albumen weight})^{0.34}$, respectively. The results of the models overall overestimated egg weight 0.098 g/d and 0.37 g/d for Hy-Line W36 and ISA-Brown hens, respectively. A significant linear bias was observed for both strain ($P < 0.01$). The assessment of the results indicates that the equations for predicting egg weight were more accurate for Hy-Line but less precise for both strains.

Keywords: egg production, genetic, laying hens, nutrition model

S2-0169 Genetic diversity of two local Yunnan chicken breeds and their relationships with Red Junglefowl

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Yunnan Province has high biodiversity, including habitats for several ancestral species of domestic chicken and has been suggested to be a center of domestication of chicken. Blood samples were collected from 60 birds of two local chicken breeds in Yunnan Province. Their complete mtDNA D-loop sequences were PCR amplified, sequenced and compared with the DNA data of five Red Junglefowl subspecies annotated in the GenBank. The results indicated that D-loop regions were 1231~1232 bp, with a base C deficiency from 859 bp site in the 1231bp haplotype. Nineteen polymorphic sites were identified across the D-loop region sequence. The eight haplotypes observed in the two breeds belonged to four previously published clades, i.e. Clades A, B, D and E. Of these clades, A and B were dominant. Clades A and B both contained three haplotypes, whereas Clades D and E both contained one haplotype. Clades A and B were clustered with *G.g.spadiceus* indicating the two clades may have originated from this subspecies. Clade E was clustered with *G.g.murgh* indicating this clade may have originated from this continental subspecies. The other haplotypes were contained in Clade D, which originated from multiple maternal origins. These data indicate that *G.g.spadiceus* has contributed more to the evolution of the two local Yunnan breeds than the other four subspecies tested. The haplotypic diversity was lower than those of breeds studied using the D-loop in other chickens of the Yunnan Province. Determining the genetic background of local breeds and their relationships with Red Junglefowl genetics could improve understanding of chicken domestication. In contrast to other chickens of the Yunnan Province, the two chicken breeds studied here had lower diversity at mitochondrial levels. Due to their possible contribution to past and recent domestication, the two breeds deserve conservation attention.

Keywords: Yunnan, chicken, Red Junglefowl, mitochondrial DNA, D-loop region

S2-0170 Estimating the effectiveness of current conservation program with genome-wide SNP from indigenous chicken breeds

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To maintain maximum genetic diversity of breeds for preserving the viability of breed, current conservation program of breed (random mating, within-family selection (R: F)) is necessary to be evaluated. In order to estimate the change of inbreeding occurrence and genetic diversity during conservation, population parameters consisting of genomic inbreeding coefficients, autozygosity derived from runs of homozygosity (ROH) were computed with SNP data from 231 samples of three chicken breeds (Beijing You Chicken (BYC), Langshan Chicken (LSC) and Baier Yellow Chicken (BEC)). Samples of each breed were collected from three generations (Gn, Gn-5 and Gn-8 for BYC and BEC; Gn, Gn-3 and Gn-5 for LSC, Gn represents current generation). After a series of filtering steps, 1286815 SNPs were available in subsequent analysis. In current generation, highest value of inbreeding coefficients was observed in BYC (0.211) followed by BEC (0.168) and the least was observed in LSC (0.0775). The inbreeding coefficients showed little decline between Gn-8 and Gn-5 ranged from 0.119 to 0.102 and then rose to 0.211 at Gn in BYC breed. Similar case was observed in BEC breed which slightly decreased from 0.0710 to 0.0308 followed by rapidly increased to 0.168. However, no obvious difference was observed among three generations of LSC. Furthermore, ROH in different cut-off lengths was analyzed to investigate the recent or ancient inbreeding occurrence. All of three breeds had rather higher proportion of short ROH, likely to reflect ancient inbreeding occurrence. Quite little number of long ROH was detected that indicated recent inbreeding occurrence could be avoided by R: F strategy. However, increment of inbreeding coefficient at Gn could be explained by many factors including sample size, estimate method etc., not only inbreeding occurrence. Generally, we could get a conclusion that R: F strategy could effectively avoid the further inbreeding occurrences.

Keywords: indigenous chickens, conservation, inbreeding coefficients, runs of homozygosity

S2- 0171 The relationship between feathering speed with body growth, maturity and feathers (hallow shaft, quill) in the Qingyuan Patridge chicken

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Qingyuan Patridge Chicken is famous breed in Guang Dong. Before that no study was conducted to know the relationship between feathering speed with body growth and feather hallow shaft. In this experiment we measured and analyzed the length of the primary and secondary feather, body weight at different stage and crown height in the rapid and slow feathering Qingyuan Patridge Chicken. Four groups were made for 200 day old chicken and 50 chickens were kept in every group (50 for each group accordingly rapid feathering male, female and slow feathering male, female). The experimental duration was 105 days. All groups were fed with same daily ration. The results showed that, the length of the 3rd, 4th primary and coverts feather including the mean feathering of rapid feathering chicken was significantly ($p < 0.01$) more than slow feathering chicken. The weight of slow feathering chicken at 42 days was significantly ($p < 0.05$) more than rapid feathering chicken. There was no significant change at other developmental stage. Feather hallow shaft (quill) of slow feathering chicken was significantly ($p < 0.01$) less than rapid feathering chicken. The research showed that in Qingyuan Patridge chicken the feathering speed has significant impact on early body weight and feathers Hallow shaft. And the result could provide certain scientific basis for Qingyuan Patridge chicken. Key words: Qingyuan Patridge chicken, feathering speed, body weight, feathers hallow shaft

Keywords: Qingyuan Patridge, feathering speed, body weight, feathers hallow shaft

S2-0172 Expression and alternative splicing of GPR1 genes in different tissues of chicken

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GPR1 is a G protein-coupled receptor that plays critical roles in eukaryotic cells. Typically, response to glucose stimulation, lipid accumulation and transmit nutrition signals to cAMP pathway. However, the alternative splicing of the GPR1 gene and its expression profile in chicken tissues and ovarian follicles have not been reported. In this study, we used rapid amplification of cDNA ends (RACE)-PCR to identify three GPR1 variants, including the full-length variant (GPR1-va1) and two alternatively spliced variants (GPR1-va2, -vb). The expression profile of GPR1 mRNA in chicken tissues and ovarian follicles were examined using quantitative real-time PCR. The result reveals that the open reading frame of the three variants cDNA is 1053, 1053 and 627 bp in length, encoding 351, 351 and 209 amino acid protein, respectively. The three variants of GPR1 show similar tissue distributions. More interesting, the expression of GPR1 gene gradually increased with follicular development. Taken together, we found three novel variants of GPR1, and the results of GPR1 expression profiling in adipose tissues and ovarian follicles suggest that GPR1 may play an important role in the lipid accumulation and progression of follicular development.

Keywords: GPR1, chicken, alternative splicing, expression profile

S2-0173 Quantitative assessment and comparison of genetic susceptibility to colibacillosis in meat-type chickens

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The aim of this study was to examine whether susceptibility to colibacillosis differs between four meat-type chicken lines, indicating genetic variation in susceptibility. This was achieved by a placebo-controlled E.coli challenge experiment and subsequent comparison of mortality rate, colibacillosis lesions and growth retardation. Commercial broilers were included and functioned as reference group. Susceptibility to colibacillosis was measured after intra-tracheal E.coli506 inoculation at 8 days of age. The experiment was ended at 17 days of age and colibacillosis lesion scoring was performed. Mortality rates in the E. coli inoculated groups were not statistically significant ($P > 0.05$) in the period of 8 to 10 days of age. From 11 to 17 days of age and from 8 to 17 days of age mortality did differ significantly between some pure lines ($P < 0.05$). Compared to their placebo inoculated chicken line mates, mean body weight of the E. coli inoculated group was significantly lower ($P < 0.05$) and uniformity in body weight was also reduced resulting in a greater standard deviation of the mean body weight. Mean body weight also differed significantly ($P < 0.001$) between chickens showing different levels of colibacillosis. Mean lesion score (MLS) differed between the chicken types. Between the four meat-type chicken lines susceptibility to colibacillosis differed, indicating genetic variation in susceptibility. The commercial broilers were the least susceptible to colibacillosis as they showed lowest mortality and MLS.

Keywords: disease resistance, broilers, Escherichia coli, breeding

S2- 0174 The differences of genetic evolution between H7N9 and H9N2 influenza Virus

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H9N2 subtype avian influenza viruses are prevalent in chickens in China, and repeatedly transmitted to humans. H9N2 influenza virus is also the gene donor for H7N9 and H10N8 viruses emerged in humans, posing threats for public health. Previously, we reported that G57 has been the predominant genotype of H9N2 in China since 2010, and provided all of their internal genes to the novel H7N9 viruses. In the present study, 26 strains of H9N2 viruses were isolated from farm chickens and live poultry markets in Beijing and Shandong provinces in 2015. Genetic analysis showed that all of these isolates were belonged to G57 genotype. To determine the genetic variation of G57 H9N2 internal genes after reassorted with H7N9 viruses, we compared the sequences of H9N2 and H7N9 viruses during 2013 to 2015 which we isolated or available in NCBI Flu database. We found that all the internal genes of the H7N9 viruses are from the G57 viruses, and several amino acid differences were identified between H9N2 and H7N9 viruses, including PB2 V139I, PB2- M570I; PB1 M171V, I397M, N694S; PA N115S, D394N, L336M; PA-X P194L; NP V239M; M2 E24D; NS2 G22R. For example, PB1 171V is present in 2% of all H9N2 viruses prevalent in China in 2013 - 2015 and in 47% of H7N9 strains. PB2-V139I located in the N-terminal NP binding region, PB2 M570I mapped to a site involved in the host 7methyl guanosine cap binding domain, PA N115S is in N-terminal region of PA which affects the polymerase activity, M2 E24D mutation resided in M2 ion channels, NS2 G22R mutation occurred in the nuclear export signal site. Noteworthy, the proportion of PA 394N among H7N9 chicken isolates is 38%, while is 83% of H7N9 human isolates. Whether this mutation related to the human adaptation should be further determined.

Keywords: H9N2, H7N9, influenza, genetic analysis

S2-0175 Integrated analysis of transcriptome and metagenome reveals the prosteatotic and protective components in a unique model of fatty liver: gut microbiota and suppressed complement system

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Non-alcoholic fatty liver disease (NAFLD) induced by excess energy consumption is globally and increasingly prevalent in both human beings and domestic animals. Goose as a descendant of migratory bird has an excellent capacity for fat deposition in its liver without overt liver injury, thus it may serve as a unique model for uncovering how the liver is protected from harmful effects associated with severe steatosis. This study was to identify the protective and prosteatotic components in this model. Using liver tissues and intestinal contents that were collected from the overfed and normally-fed geese at different time points (day 7, 14 and 19 of overfeeding), we performed an integrated analysis of liver transcriptome and gut microbial metagenome. The results showed that a total of 458 (267 upregulated), 875 (536 upregulated) and 1,733 differentially expressed genes (946 upregulated) were identified at day 7, 14 and 19 of overfeeding, respectively, indicating that fatty liver transcriptome, initially featuring a 'metabolism' pathway, was later joined by 'cell growth and death' and 'immune diseases' pathways. Simultaneously, gut microbiota responded to overfeeding in harmony with the liver response, as microbial and hepatic genes affected by overfeeding shared multiple pathways. This microbial response varied with different intestinal segments and overfeeding time. Remarkably, the complement system, an inflammatory component, was comprehensively suppressed in fatty liver, which was partially attributable to increased blood lactic acid from enriched *Lactobacillus*. Our findings suggest that gut microbes and their host respond to excess energy flux as an organic whole, and both are gradually modulated to promote the development of goose fatty liver in a coordinated manner. Moreover, suppressed complement system, as a protective component in goose, may prevent the progression of plain steatosis into steatohepatitis.

Keywords: goose, gut microbiota, inflammation, metagenome, non-alcoholic fatty liver disease, transcriptome, complement system

S2- 0176 Using CRISPR to edit chicken sex determination candidate gene DMRT1

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DMRT1 is highly conserved gene in vertebrates and homologous to the two genes (doublesex and mab23) associated with the male development in the flies and worms. DMRT1 encodes transcription factors and it has zinc finger DNA-binding domain called DM domain. So we call it DMRT1. The expression of DMRT1 in male gonads is higher than female gonads in chickens, mammals, reptiles and fish. DMRT1 in Poultry is on the Z chromosome, it is one of the most important sex determination candidate genes. In recent years genome editing technology CRISPR becomes popular, whose core of the system is CRISPR/Cas. The RNA (sgRNA) complementary to the target sequence guides the DNA enzymes Cas to cut the target sequence. CRISPR has a great advantage comparing with traditional gene editing method, which is applied to gene function transformation in varieties of organism, but the application in the chicken is rare. Using the technology to edit chicken DMRT1, expect to establish platform where editing chicken gene with CRISPR, and provide an effective method for chicken DMRT1 gene function research, to lay a good foundation for the poultry related gene editing. The experiment materials are chicken DF-1 cells. Firstly, sgRNAs that target to DMRT1 were designed; Then to build sgRNA expression vector and extract plasmid, Translate PCR product into DH5 α competent cells, select monoclonal bacteria liquid to sequence. Finally to extract respectively sgRNA and Cas9 expression plasmid, cultivate chicken DF-1 cell and transfect sgRNA and Cas9 expression plasmid to DF-1 cell. The results are as follows: Sequencing results show that design of sgRNAs targeted chicken DMRT1 is successful, sgRNA liposome and Cas9 nucleic acid enzyme expression plasmid were transfected into chicken DF-1 cell successfully.

Keywords: CRISPR, chicken DMRT1, gene editing, chicken DF-1 cell

S2- 0177 Identification of candidate genes for chicken early- and late-feathering

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Previous studies suggest that prolactin receptor (Prlr) is a potential causative gene for chicken early- (EF) and late-feathering (LF) phenotypes. In this study, we evaluated candidate genes for this trait and determined the expression of three genes, including Prlr, sperm flagellar protein 2 (Spef2), and their fusion gene, in the skins of 1-day-old EF and LF chicks using RT-qPCR. Data indicated that Prlr expression in the skin did not show significant difference between EF and LF chicks, suggesting Prlr may not be a suitable candidate gene. In contrast, Spef2 expression in the skin displayed a significant difference between EF and LF chicks ($P < 0.01$), suggesting that Spef2 may be a good candidate gene for chicken feathering. Moreover, dPrlr/dSpef2, the fusion gene, was also a good candidate gene as it was only expressed in LF chicks. However, the expression of the fusion gene was much lower than that of Prlr. Additionally, using strand-specific primers we found that the fusion gene was transcribed in two directions (one from dPrlr promoter, another from dSpef2 promoter), which could result in the formation of a double strand RNA. In conclusion, both Spef2 and the fusion gene are good candidate genes for chicken feathering, but Prlr is not. The research on the function and regulation of the candidate genes will help elucidate the molecular basis of the chicken feathering trait.

Keywords: chicken, endogenous retrovirus, feathering, Prlr, Spef2

S2-0178 Selection for improved gait in poultry—finding the correct phenotype

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Breeding success in the broiler chicken has been accompanied by gait problems which are detrimental to productivity and welfare. Although these gait issues have not been reported to the same extent in Pekin ducks, there is concern that such problems will manifest if the duck continues on its current selection trajectory. In order to understand how changes in morphology due to selection have affected gait in both species, divergent lines were objectively assessed for gait using a pressure platform (12 birds per line at 3 ages). The broiler chicken was compared to the slower growing layer chicken and the Pekin duck to its slower growing ancestor, the mallard. After gait assessment, the leg bones were scanned by computed tomography to measure morphological changes which have occurred due to selection for high growth and meat yield. Results were analysed by ANOVA, accounting for age and sex. During walking, heavy lines displayed a wider stance ($P<0.001$) and spent more time supporting their mass on both feet ($P<0.001$) than their lighter conspecifics. The foot angle while walking differed between lines ($P<0.001$); both duck lines rotated their feet internally whereas the layer chickens' feet were aligned with the direction of travel. Conversely the broiler chicken rotated its feet externally. Morphologically, the main differences between species were in bone curvature; the tibiotarsus curved more laterally in ducks than in chickens, which may be a swimming adaptation acquired prior to domestication. Intense selection for economic traits has altered gait in similar ways in both species. To improve gait in poultry, greater breeding success may be achieved by focussing on those components of gait which have changed through selection, rather than using a subjective overall visual gait score. Furthermore, in both species, adaptations for pre-domesticated life may have affected the ability with which the selected lines have accommodated their gait to other morphological changes.

Keywords: gait, welfare, Pekin duck, broiler chicken, leg, bone

S2-0179 Identification of sperm motility related proteins in chickens using iTRAQ-based proteomic analyses

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The egg and meat quality and disease resistance ability of China domestic chicken breeds are excellent, but their fecundity is lower. The aim of this study was to identify some key proteins that affect sperm motility to enhance understanding the mechanisms and underlying the fecundity of cocks. A total of 200 Beijing-You males with pedigree information was randomly selected from a conservation population and housed in the same environment. Twenty-five low sperm motility and 25 normal ones were identified by semen quality detection from 43 to 47 weeks of age. Mating trial was performed at 48 weeks of age to verify the fecundity. At 52 weeks of age, the left testes of these birds were obtained for tissue sectioning to observe the size and measure the testis parameters such as Johnsen score. Based on the observed traits, three full-sib couples of high and low sperm motility were selected for iTRAQ and for further GO (gene ontology) and Pathway analysis. Results: Total number of 4266 proteins was detected in which 1945 proteins were annotated and 2325 proteins were uncharacterized. During all intercomparisons of the three full-sib group, 70 down-regulated and 90 up-regulated proteins were identified in the birds with low sperm motility. Glutathione S-transferase (GST), Aspartate amino transferase (AspAT), and Histone H2A were the most high-expressed proteins while DNA-Damage binding Protein (DDBP) and Protein Tyrosine Phosphatase ReHZptor Type G (PTPRG) were the most down-regulated. The GO analyses reveals that the differentially expressed proteins were mainly enriched in Purine and pyridine metabolism and protein transport pathways. Western Blotting further verified the express pattern of GST, AspAT and Histone H2A. Based on these results, AspAT, GST, DDB2, and histone H2A were proposed as important candidate proteins which affect chicken sperm motility. Pathways of GnRH signaling, Purine and pyridine metabolism and protein transport were found to be the main pathways.

Keywords: Beijing-You chickens, sperm motility, iTRAQ, proteomics

S2-0180 Relationship between body composition and production traits in a free-range broiler stock

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The objective of this work was to establish phenotypic relationships among some production traits (feed conversion rate, feed consumption-FC, residual feed consumption, relative weight gain, weight gain-WG and live weight) and body composition traits in a free-range broiler population (Campero-INTA). Ninety-six males in individual cages between 49 and 79 days of age were used. Weight and feed consumption was recorded weekly. Forty-four broilers were slaughtered at 44 days of age to estimate the initial body composition. All caged birds were slaughtered at 79 days of age and the following weights were recorded: live, carcass (CW), viscera (VW), abdominal fat (AFW), breast, thigh (TW) and feathers. Correlation coefficients between traits were obtained and regression analysis was performed for the evaluation of the influence of production traits over the slaughter proportion gain and slaughter weight gain of the body components. Any proportion gain between slaughters was well explained by the measured and calculated production traits. Only the gain of two body components (CW, TW) was well explained by some of them (WG, FC). There was little association between body components and production traits when they were analyzed independently. But when proportions of body components were correlated with all production traits by a canonical correlation, AFW and VW proportions were the most important variables, with opposite signs, in the association. Some observed correlations encouraged us to further study these traits for selection criteria.

Keywords: broiler, body composition, free range

S2-0181 Fine mapping of a QTL affecting growth and muscle mass on chicken chromosome 4 in a cross between New Hampshire and White Leghorn

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In our previous study, a QTL affecting growth in chickens was found on chromosome 4 (from 61.5 to 88.4 Mb, Nassar et al., 2015, *Animal Genetics* 46: 441-6) using a reciprocal F2 cross between New Hampshire (NHI) and White Leghorn (WL77). In order to reduce the QTL confidence interval, an advanced intercross line has been generated from the initial F2 mapping population. Here we investigate nine single nucleotide polymorphism (SNP) markers located in the target QTL region and genotyped 188 males of generations F10, F11 and F12. Growth traits from hatch until 20 weeks and body composition traits at 20 weeks were used to perform association analysis. We reduced the size of the confidence interval of the QTL region from 26.9 Mb to 3.4 Mb. The number of genes in the confidence interval drops from 292 in the original region to 30 in the fine-mapped region. In addition, sequence variants were investigated between NHI and WL77 using 60K-SNP, 600K-SNP Chip and DNA sequencing of the parental lines. Using this information we detected a missense SNP within the ADGRA3 gene, a frame-shift deletion in the gene ENSGALG00000014401 with unknown function and synonymous variants in additional five genes. Since the QTL we described here has been mapped in crosses between different chicken breeds, the candidate genes we provided could also contribute to understand the genetic determinants for meat production in other chicken breeds.

Keywords: chicken, advanced intercross line, growth, muscle mass, fine mapping

S2- 0182 Genome wide association analysis for avian influenza resistance in chicken

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Highly Pathogenic Avian Influenza (HPAI) has a devastating impact on the poultry industry with high mortality, reduced egg production and mandated euthanization of infected flocks. Within the last 4 years, two HPAI outbreaks have affected egg production flocks in the American continent; a H7N3 outbreak in Mexico in 2012 that caused 70-85% mortality and a H5N2 outbreak in the US in 2015 with over 99% mortality. Blood samples were obtained from survivors of each outbreak plus age and genetics matched non-affected controls. DNA from 247 survivors and 237 controls was SNP genotyped using the Affymetrix 600K Chicken SNP array. Of these, 55 were removed by quality control. 420,000 high quality, segregating SNP were identified across all samples. Genetic differences between survivors and controls were analyzed using PLINK (Purcell et al 2007). Pruning with an r-square of 0.2 identified 12930 approximately independently segregating SNPs, which were used for multi-dimensional scaling (MDS) and adjustment for multiple testing. A logistic regression model with the effects of year, location, first two MDS components and an additive SNP effect was used to identify SNPs associated with resistance. The analysis identified significant or suggestive associations with AI survival for SNPs on chromosomes 1, 3, 4, 8, 11 and 19. The Mexico and US outbreaks were caused by different strains of the virus, with different levels of mortality, so an analysis within outbreak was also performed, resulting in the identification of different genomic regions. The identification of genomic regions that influence survival following HPAI infection can potentially identify candidate genes involved in viral resistance mechanisms, increase understanding of viral pathogenesis and provide targets for genetic selection for resistance to HPAI.

Keywords: avian influenza, highly pathogenic avian influenza, whole genome association

S2-0183 Genetic potential of egg production in Jing Brown Layers

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Prolonged production cycle of laying hens benefit for several reasons, such as more sustainable management, spread the pullet cost over a higher production, and reduce labor and time for cleaning and disinfection of the house between production cycles. Therefore, the present study was conducted to investigate the potential of extending the laying period to 80 weeks of age in brown layer. A total of 1082 recorded individuals from a Jing brown pure line were studied. The estimates of heritability were analyzed by the average information restricted maximum likelihood algorithm based on mixed animal model. The average age of first egg (AFE) was 137 days, while the heritability was 0.58. The egg numbers to 80 weeks (EN80) was 377 on average and its heritability was 0.09. The individual laying performance up to 80 weeks showed that 202 layers had more than 95% of lay, which accounts for 18.67%. There were 452 individuals with 90 to 95% rate of lay, which takes 41.77%. And the rest were 226 birds with 85 to 90% rate of lay (20.89%), 87 with 80 to 85% rate of lay (8.04%), and 115 hens with less than 80% of laying rate (10.63%). These results suggested the higher genetic potential of the laying performance from the beginning of production until 80 weeks of age in Jing brown layers. As the heritability of egg number was relatively lower, thus, a second selection based on data till 80 weeks would increase the selection accuracy, which contribute to further improve performances by extended production cycle on a persistent level.

Keywords: layers, genetic potential, egg numbers, heritability

S2-0184 Genome resequencing and bioinformatic analysis to elucidate the molecular mechanisms that underlie residual feed intake (RFI) in chickens

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Improving feed efficiency (FE) is a major goal in poultry production in order to reduce production costs and increase profitability. Residual feed intake (RFI) is an accurate indicator of FE that is being accepted as an alternative measure for FE. Therefore, genome resequencing was performed between low-RFI (LRFI) and high-RFI (HRFI) chickens to understand the genetic components underlying the RFI. Method: The RFI of Beijing-You and Cobb was measured from d 56 to d 98 and from d 28 to d 42 respectively. The DNA samples from chickens with extremely high ($n = 48$) or low ($n = 48$) RFI were pooled into three pools, and both of the breeds were performed the same treatment. Illumina sequencing technology and reference based assembly on Red Jungle Fowl genome sequences were used. Results: Genome resequencing of each pool reached about 20x coverage. The number of SNPs was 8,505,214 and 8,479,041 in LRFI and HRFI genome of Beijing You, and the number of SNPs was 8,352,008 and 8,372,769 in LRFI and HRFI genome of Cobb. The 8110 reliable marker SNPs included 1380 genes in Beijing You and 785 genes with 1882 SNPs in Cobb were chosen for further analysis. Biological processes enriched with genes suggested that genetic variation in RFI was driven by Jak-STAT signaling pathway, regulation of cell proliferation and apoptosis, gut development and transcription in the Beijing You. However, the biological processes such as Calcium signaling pathway, GnRH signaling pathway, Vascular smooth muscle contraction, lipid transport, polysaccharide metabolic process and translational initiation might contribute to the RFI in the Cobb. Conclusion: This study provides novel insights into genomic differences between LRFI and HRFI chickens, which indicate that developmental growth, metabolism, genetic information processing may influence the RFI in poultry, however, the genes or biological processes that involve in RFI are difference between Beijing You and Cobb chickens.

Keywords: genome resequencing, residual feed intake, molecular mechanisms, chickens

S2-0185 Effects of the TGF- β signaling pathway on the differentiation of chicken embryonic stem cells into male germ cells

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The objectives of the present study were to screen for key gene and signaling pathways involved in the production of male germ cells in poultry and to investigate the effects of the transforming growth factor beta (TGF- β) signaling pathway on the differentiation of chicken ESCs into male germ cells. The ESCs, PGCs, and SSCs were sorted using flow cytometry, and ESCs, PGCs, and SSCs gene expression levels were detected by RNA sequencing (RNA-seq) technology. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) and WEGO were used to explore the transcriptomic differences among the three cell types and to screen for key gene and signaling pathways, and the results were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR). Male chicken ESCs were induced using 40 ng/ml of bone morphogenic protein 4 (BMP4), which is a member of TGF- β superfamily. The effects of the TGF- β signaling pathway on the production of chicken SSCs were confirmed by morphology, qRT-PCR, and immunocytochemistry. One hundred and seventy-three key genes relevant to development, differentiation, and metabolism, and 20 signaling pathways involved in cell reproduction, differentiation, and signal transduction were identified by RNA-seq. Likewise, the qRT-PCR and RNA-seq results were consistent with TGF- β signaling pathway gene expression levels. The germ cells formed agglomerates and increased in number 14 days after induction by BMP4. During the induction process, the ESCs, Nanog, and Sox2 marker gene expression levels decreased, whereas expression of the germ cell-specific genes Stra8, Dazl, integrin $\alpha 6$, and c-kit increased. The results indicated that the TGF- β signaling pathway participated in the differentiation of chicken ESCs into male germ cells. The present paper provides preliminary information that will aid in elucidating the network that regulates the differentiation of male germ cells in chickens and the discovery of germ cell cytogenesis.

Keywords: embryonic stem cells (ESCs), primordial germ cells (PGCs), spermatogonial stem cells (SSCs), TGF- β signaling

S2-0186 Molecular characterization of two indigenous native chicken breeds using microsatellite markers

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A study was conducted to characterize two native Indigenous chicken breeds namely, Aseel and Kadaknath using 14 microsatellite markers. All the markers except MCW048 was found as polymorphic where polymorphic information contents were ranging from 0.21 to 0.87. The percentage of polymorphic markers in both the population was 92.88. A total of 6 alleles were observed across the markers in these two breeds. The observed number of alleles (Na) in Aseel and Kadaknath breeds was 4.1 and 4.2 while effective number of alleles (Ne) was 3.3 and 3.2, respectively. The average observed heterozygosities were 0.72 and 0.61 in Aseel and Kadaknath breeds, respectively while expected heterozygosities were 0.65 and 0.64, respectively. The Nei's heterozygosity was 0.64 and 0.62 in Aseel and Kadaknath breeds, respectively while Shannon's information index were 1.2 and 1.17. The ADL102, MCW049, MCW044 and MCW059 markers showed heterozygosity excess in Aseel breed whereas ADL102, MCW014, MCW049, MCW044 and MCW059 markers had heterozygosity excess in Kadaknath breed. The remaining markers except MCW048 showed heterozygosity deficiency. The Nei's genetic distance between these two populations was 0.06. From the results, it is concluded that microsatellite markers may be extensively used to characterize native chicken breeds showing genetic diversity within and between breeds.

Keywords: diversity, microsatellite, native chicken

S2-0187 Study on the relationship between eggshell color and eggshell quality in *Anas platyrhynchos*

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Eggshell quality has become a principal concern of commercial egg production. This study investigated the relationship between eggshell color and eggshell quality for the sake of improving egg quality and poultry breeding. A total of 600 eggs used in this experiment were randomly selected from 2 sets of eggs allocated by eggshell color to 'blue' and 'white' varieties on a commercial farm. The blue eggs (n=300) and white eggs (n=300) were randomly allocated to ten groups (each with 30 eggs), respectively. The eggshell color, eggshell thickness and eggshell strength were measured. Quantification of eggshell color could be implemented by measuring the chromatic values of the eggshell at three points with HunterLab MiniScan EZ (4500L) hand-held colorimeter that works on the L*a*b* color space system. Principal component analysis was applied to evaluate the eggshell color. The correlations between eggshell color and eggshell thickness, eggshell strength were indicated by Pearson correlation coefficient. Comprehensive evaluation of blue and white eggshell color respectively represented in the following function: $ZFB = 55.585\% \cdot FB1 + 32.845\% \cdot FB2$; $ZFW = 55.252\% \cdot FW1 + 35.560\% \cdot FW2$. The results demonstrated that eggshell color has a significant effect on eggshell thickness and eggshell strength ($P < 0.05$) which were higher in blue eggs than white eggs. There were significant correlations between eggshell color and eggshell strength, eggshell thickness ($P < 0.05$). This study established two comprehensive evaluation functions of eggshell color to interpret the relationship between eggshell color and eggshell quality. These models may have important significance for practical application. It was concluded that eggshell quality could be assessed by the eggshell color. Consequently, it recommends that blue eggs should be preferably selected for commercial egg production and poultry breeding of *Anas platyrhynchos*.

Keywords: eggshell color, eggshell quality, *Anas platyrhynchos*, principal component analysis

S2- 0188 Identification of differentially expressed genes and pathways for intramuscular fat metabolism between breast and thigh tissues of chickens

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Intramuscular fat (IMF) is one of the important factors influencing meat quality, however, for chickens, the molecular regulatory mechanisms underlying this trait have not yet been clear. In this study, a systematic identification of differentially expressed genes (DEGs) and molecular regulatory mechanism related to IMF metabolism between Beijing- you chicken breast and thigh at 42 and 90 days of age was performed. IMF contents, GO terms, and KEGG pathways were analyzed. The results showed that both IMF contents in thigh at 42 and 90 d were significantly higher ($p < 0.05$) than those in breast. 1581 common known DEGs and 80 DEGs related to IMF metabolism were identified between the thigh and breast at 42 and 90 d. It was found that the expression levels of THRSP, PLIN, LDLR, PPARG, LPL and FABP4, which participate in the processes of lipid biosynthesis, and CYP8B, FABP3, CPT2 and PPARGC1A, which participate in the processes of lipolysis, had up-regulated in thigh compared to breast. In addition, based on KEGG pathway analysis of DEGs, it was found that in addition to pathways affecting lipid metabolism (pathways for PPAR signaling pathway and fatty acid metabolism), cell junction-related pathways (ECM-receptor interaction, focal adhesion, regulation of actin cytoskeleton), which play a prominent role in maintaining the integrity of tissues, could contribute to the IMF metabolism. The results of this study identified potential candidate genes associated with chicken IMF metabolism, and imply that IMF metabolism between thigh and breast in chicken is regulated and mediated not only by genes and pathways related to lipid metabolism, but also by others involved in cell junctions. These findings establish the groundwork and provide new clues for deciphering the molecular mechanisms underlying IMF deposition in poultry. Further studies at the translational and posttranslational level are now required to validate the genes and pathways identified here.

Keywords: intramuscular fat, DEGs, pathways, breast and thigh, chickens

S2- 0189 Exploring the molecular mechanism of abdominal fat accumulation in capons using gene expression profiling

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Capons are male chickens whose testes have been surgically incised. Caponization leads to a significant reduction in androgen levels but shows a significant increase in fat accumulation compared to intact male chickens, however, its effect and the molecular mechanisms underlying it are incompletely understood in avian species. Therefore, investigation of the influence of androgen status on fat accumulation in the chicken will provide insights into this process. In this study, RNA sequencing technology was used to analyze the gene expression profiles of abdominal fat from capons and intact male chickens. After caponization, serum testosterone content decreased dramatically and abdominal fat content exhibited a greater increase in the capon group compared with the control ($P < 0.05$) at different ages. Testosterone is present in abdominal fat and the capon group exhibited a lower testosterone content in abdominal fat than the control ($P < 0.05$). Through pathway and gene ontology, it was found that in addition to genes involved in lipid metabolism and steroid biosynthesis, cell junction-related pathways (ECM-receptor interaction, cell adhesion molecules (CAMs), focal adhesion) were also highly enriched, maybe could contribute to the fat deposition. Eleven genes (SCD, FABP7, APOA1, RXRG, FADS2, MSMO1, SQLE, MGLL, PTGDS, HPGDS, CYP2D6) that are mainly involved in pathways for lipid metabolism regulation, such as PPAR signaling pathway, arachidonic acid metabolism, fatty acid metabolism, and steroid biosynthesis signaling pathway were selected for validation by a quantitative real-time polymerase chain reaction (qRT-PCR) analysis, the qRT-PCR results are consistent with the general expression patterns of those genes from the Illumina sequencing. This suggested a role for these pathways in the fat accumulation of capons that might increase our understanding of the capon's biological mechanism.

Keywords: abdominal fat accumulation, molecular mechanism, capons, gene expression profiling

S2- 0191 Gene expression profiling of the duodenum, jejunum and ileum of village chickens naturally infected with *A. galli*

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This study investigated differential gene expression profiles of the *A. galli* infected jejunum, ileum and duodenum segments of the small intestines of village chickens using RNA-seq strategy. Total RNA isolated from duodenum, jejunum and ileum of 2 infected and 2 non-infected village chickens were sequenced using Illumina HiSeq2500 to generate between 3,908,924 and 3,994,946 reads. An average of 83.50% quality controlled reads mapped to the reference chicken genome (*gallus.galgal4.74*). The multidimensional scaling plots revealed tissue dissimilarity of genes expression patterns by clearly separating the anatomical sections of the small intestine. The different sections differed in the quantitative composition of the genes expressed in response to *A. galli* infection. A total of 76, 99 and 78 differentially expressed genes were identified in duodenum, jejunum and ileum sections respectively. Of these, 33, 15 and 18 differentially expressed genes were identified in duodenum, jejunum and ileum sections of KZN chickens respectively. A total of 31 and 10 genes were co-expressed in all the sections of chickens from Limpopo and KZN respectively. Analysis of genes uniquely expressed in the different tissue types gives a better understanding of the unique immunological attributes of the various tissues that are involved in parasite colonization. Predominant biological processes terms observed were cellular process, biological regulation and response to stimulus. KEGG pathway analysis showed variation in the different tissues of the small intestine. Pathways such as the Linoleic acid metabolism and Arachidonic acid metabolism were common in the two provinces but enriched for in different sections. Overall, this study suggests region-specific expression patterns and functional annotation of genes involved in response to *A. galli* infection in chickens. Such information could aid in better understanding of host-parasite interactions in development of effective control strategies.

Keywords: *A. galli*, village chickens, RNA-seq, gene expression

S2-0192 Analysis on changes of hypothalamic POK/PcG/TRG pathway transcription during puberty onset in Wenchang chicken

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This experiment was conducted to investigate the role of POK/PcG/TRG pathway in chicken puberty onset. The Wenchang chicken breed was used as the material. The comb size, ovary weight, oviduct length and follicle size for hens were measured from 5 to 17 weeks. qRT-PCR was performed to analyze the hypothalamic expression changes of POK/PcG/TRG pathways. The results showed that 1) the ovary and oviduct developed slowly and the follicles kept primitive in early period of development in Wenchang chicken. This period would last about 12 weeks. Then, the gonad tissues developed explosively, the ovary weight and oviduct length of 13 weeks increased 125% and 120% respectively compared to that of 12 weeks, and accompanied by emergence of pre-hierarchical follicles. Based on these, the crucial “timing” for transition from juvenile to puberty onset for Wenchang hens was determined as age of 13 weeks. 2) qRT-PCR test revealed the relative expression levels of BCL6 and ZBTB7A were lower before puberty onset, and BCL6 level increased significantly ($P < 0.01$) after puberty onset, but the significant changes for ZBTB7A occurred at 15 week, later than BCL6. The relative expression levels of YY1 and EED were higher before puberty onset, and decreased significantly ($P < 0.01$) after puberty onset. However, the decrease only kept 2–3 weeks, and then recovered to higher levels from 15 weeks. The expression levels of Oct-1 were lower before puberty onset, and increased significantly ($P < 0.01$) after puberty onset. The higher level of Oct-1 could last to the age of first laying. But the relative expression levels of TTF1 decreased significantly ($P < 0.01$) after puberty onset, which was contrary to previous reports. It suggested the presence of other pathways. These results indicated that POK/PcG/TRG regulating pathway was involved in chicken puberty onset.

Keywords: Wenchang chicken, puberty onset, hypothalamus, gene expression

S2-0193 Gga-miR-181a modulates ANP32A expression and Inhibits MDCC-MSB1 cell migration and proliferation

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Marek's disease (MD), a highly contagious T-cell lymphoid neoplasia disease of chickens, causes huge economic losses to the poultry industry. It is the only one tumor disease which can be prevented by vaccine, therefore MD is considered to be an excellent model to study the pathogenesis of virus-induced cancer. Recently, abundant evidences have verified that miRNAs are regulators in the process of neoplastic transformation. In our previous study on miRNome analysis of MDV-induced lymphoma in chicken, we found that gga-miR-181a was down-regulated drastically in MDV-infected spleens. To further investigate the role of gga-miR-181a in MDV-induced lymphomagenesis, we performed cell proliferation and migration assay, and the results suggested that gga-miR-181a suppressed the proliferation and migration of MDV-transformed lymphoid cell (MSB-1). Subsequently, luciferase reporter gene assay revealed that acidic nuclear phosphoprotein 32A (ANP32A) was a functional target gene of gga-miR181a. Real-time PCR and western blot assay were conducted to detect the effects of gga-miR-181a on mRNA and protein expression of ANP32A. The results showed that the mRNA and protein levels of ANP32A were down-regulated in gga-miR-181a mimic group at 48 and 96 hours post transfection, respectively, indicating that ANP32A was modulated by gga-miR-181a. All the results suggested that gga-miR-181a could be an inhibitor in MSB-1 cell proliferation and migration. ANP32A was a direct target gene of gga-miR-181a and they were involved in MD lymphoma tumorigenesis.

Keywords: chicken, Marek's disease, gga-miR-181a, ANP32A

S2-0194 The genetic diversity of qualitative traits of Kokok Balenggek chicken under ex situ conservation in West Sumatera Indonesia

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Kokok Balenggek chicken is one of animal genetic resources (AnGR) derived from west Sumatera that developed in endemic area. They are unique as they have a very nice song with multi-level sound. The identification of genetic diversity of indigenous chicken is very important for animal conservation. Characterization of AnGR encompasses all activities associated with the identification, qualitative and quantitative description. An experiment was conducted to identify the genetic diversity of qualitative traits of Kokok Balenggek chicken in ex situ area in Solok, West Sumatera. A total number of 233 Kokok Balenggek chicken (102 female and 121 male) were characterized for qualitative traits. The qualitative traits base on color of feather, plumage, flick feather, feather pattern, shank colour, and comb types. Data were analyzed using formulas to identify the allele frequency and the genetic variability. The method were used to analyze the frequency of autosomal and sex-linked of the genes, feather pattern and heterozygosity. The result indicated that the predominant of allele frequency of qualitative traits of Kokok Balenggek chicken are coloured (i) 0.82, columbian (e) 0.53, barred feather (B) 0.64, golden flick feather (s) 0.73, yellow shank coloured (Id) 0.57 and single comb (p) 1.00. Based on the type of plumage color of Kokok Balenggek chicken have shown predominantly on Biriang (30.39%). According to the rate of heterozygosity value of Kokok Balenggek chicken, genetic variability varied within 34.95%. In conclusion, the Kokok Balenggek chickens show genetic diversity on the qualitative traits. The further study on their quantitative traits and the molecular composition are needed to complete a set of characterization of Kokok Balenggek Chicken

Keywords: diversity, qualitative traits, kokok balenggek, allele frequency

S2-0195 Application of entropy analysis to detect chicken genome regions associated with performance traits

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Over last years, some new methods (as alternative to classical approach - genome wide association studies) have been applied to genomic data in order to identify SNPs contributing to complex traits. The entropy analysis is employed in the present study. Data contains records of 862 individuals genotyped using the 60K Illumina iSelect chicken array providing information about 57636 SNPs. Finally, after data editing, 855 individuals (with 43146 SNPs) were analyzed. The following traits were recorded: body weight at 39 days, body weight at 46 days, body weight changes between 39-46 days, feed intake between 39-46 days, feed conversion ratio. Prior to the analysis, the continuous performance traits are classified into quartiles. The following parameters were estimated: entropy of trait, conditional entropy and mutual information. The most informative SNPs are located on chromosome 1, 2, 4, 8, 12, and Z. Large mutual information was registered for SNPs located nearby. Clusters of the most informative pair of SNPs connected with all recorded traits were located on chromosome: 1, 2, 3, 4, 6, 12, 20, and Z. Additionally, SNPs interacting with all analysed subsets of SNPs were observed. * The study was supported by the European Union Seventh Framework Programme (FP7/2007-2013) as part of the ECO-FCE project under grant agreement No. 311794.

Keywords: SNPs, chicken, entropy analysis

S2-0196 Identification of Copy number variations at candidate loci associated with egg-laying performance in Chinese Xinhua chicken

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In recent years, a mass of duplicated and deleted DNA sequence were found in human and animal genomes following the prevalence of employing high-throughput sequencing and high-density SNP array. The formation of those CNVs are the result of the evolution of species. In chicken, those discovered CNVs were generally mapped and could be easily obtained from many papers. But the next work further studying the function of those discovered CNVs remain many technical problems unsolved. The objective of this study was to identify some CNVs relating to the egg production and quality traits and elucidate the mediated mechanism of those related genes. In the current study, we selected 17 loci according to the published CNV map and used CNVplex to detect their copy numbers in Xinhua layers. Only 2 loci were identified with an obvious copy number distribution. Next, Association analysis were conducted and showed that one locus located upstream of TMEM86A gene was significantly associated with the largest clutch size ($p=0.008$) and the other one located in the first intron of PCDHA2 gene was significantly associated with the first-egg age ($p=0.001$). We speculate that the two loci were involved in the development of the ovary of chicken, the studies of the function of related genes are in progress. We hope this finding could be a feasible attempt for improving the genetic progress in egg production and quality in chicken breed industry.

Keywords: CNVs, CNVplex, egg production and quality traits

S2- 0198 GDF9 mRNA expresses sex-differentially in chicken embryonic gonad

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Growth differentiation factor 9 (GDF9), one member of transforming growth factor- β (TGF β) superfamily, plays an important role in mammalian ovarian development. Ovarian development is a continual progress from embryo to sexually mature adult. Are factors such as GDF9, which control ovary growth also participate in embryonic gonad development? In order to figure out that, we monitored GDF9 mRNA expression in embryonic gonads of both sexes by real time quantitative PCR (qPCR). 300 commercial Hy-Line brown fertile eggs were incubated at 38°C and dissected in every two days from day10.5 to day20.5. RNA were extracted from male and female gonad pools (n=4-5), and reverse-transcribed into cDNA, which were used as templates for qPCR. We found that GDF9 mRNA expressed in a sexually biased manner (significantly higher in female), indicating its role in female gonadal development. GDF9 maintained a relatively low expression in male gonad along the investigating time. In female gonads, GDF9 mRNA level rose gradually as embryo grew, and elevated dramatically at day20.5, corresponding to the time when vertebrate oogenesis began. Differential expression patterns of GDF9 between female and male suggest its contribution to chicken embryo gonadal development.

Keywords: GDF9, chicken, embryo, sex

S2- 0199 Resequencing of Emei black fowl genomes identifies patterns of artificial selection

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In this study, we reported the analysis of genome-wide genetic variation based on whole genome sequencing of six Emei black fowls with an average of 18.77-fold coverage depth. Blood and DNA samples were gathered and extracted from six Emei black fowls (Leshan, Sichuan Province, China), and sequenced based on Illumina HiSeq PE150 platform. A total ~133Gb high quality paired-end reads were mapped to the chicken genome assembly using the BWA software. SNP calling was performed on a population scale using the Bayesian approach as implemented in the package SAMtools. Functional enrichment analysis of Gene Ontology, Pathway and InterPro domains was performed using DAVID web server. Principal Component Analysis with the population scale SNPs was performed using the package EIGENSOFT4.2. In addition, the genome sequence data of red jungle fowls with sample accession number PRJNA241474 downloaded from NCBI was also analyzed in this study. Comparison of the genomes between Emei black fowls and red jungle fowls, a total 6.60 Mb SNPs was detected in the genome of Emei black fowl, among which, 0.57 Mb SNPs were novel SNPs. Meanwhile, we also identified 337 genes under selection in Emei black fowl, and 29 of them were involved in 14 domain terms ($P < 0.05$), such as ‘calcium channel activity’ and ‘Caldesmon and lymphocyte specific protein’. Interestingly, we also found genes such as FSH β , ESRRB, TSHR and IGF2BP3 were under selection, that FSH β and ESRRB may be closely related to the low broodiness and high egg production in Emei black fowl, TSHR and IGF2BP3 may be closely related to the high growth rate of Emei black fowl. In this study, the genetic relationships between Emei black fowl and red jungle fowl will provide an important resource for further improvements of this important livestock species. The work presented here may also serve as a typical demonstration for future deciphering the genomic differences shaped by the artificial selections.

Keywords: chicken, resequencing, genomes, SNPs

S2-0200 Karyotype analysis of transgenic chickens

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This study investigated the influence of exogenous gene P53 on the chromosome karyotype and the location of the gene P53 attached on chromosome in the transgenic chickens. The transgenic roosters with exogenous gene P53 were used as the experimental group, and the same breed with non-transgenic males were used as the control group. Chromosome specimens in metaphase were completed through the peripheral blood lymphocytes culture method and bone marrow method. Fifty cells with clearly dispersed phase were randomly selected to measure the number of chromosomes under 100x oil immersion. The top 10 pairs of large chromosomes including a pair of sex chromosomes were observed to determine the chromosome morphology by measuring relative length and arm ratio and centromere index in each chromosome in the transgenic and non-transgenic chickens. The results showed that the chromosome numbers of cocks in the experimental and control group are in line with $2n = 78$ which both contained 10 pairs of large chromosomes and 29 pairs of tiny chromosomes with basic telocentric. The karyotype in the two groups contained the 38 pairs of autosomes and one pair of sex chromosome ZZ. Chromosome 1, 2 and the sex chromosome ZZ are the central centromere (m) chromosome; Chromosome 3 are the telocentric (t) chromosome; Chromosome 4 are the Asian central centromere (sm) chromosome; The chromosome from NO.6 to 10 are telocentric (t) chromosomes. The results concluded that there was no significant difference in chromosome morphology between the transgenic and non-transgenic chickens, which indicated that the exogenous gene P53 has no effect on the chromosome karyotype of chickens. The further study should be evaluated to find the location of the exogenous gene P53 attached on chromosome in the transgenic chickens.

Keywords: transgenic chickens, non-transgenic chickens, peripheral blood lymphocytes culture method, bone marrow method, karyotype analysis

S2-0201 Digital gene expression profile analysis of pituitary tissue in Ningdu Huang cocks before and after puberty

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To study the gene expression profile differences between pituitary tissue between early maturity groups and late maturity groups of Ningdu Huang cocks before and after puberty, four cDNA libraries, which including 32 d pre-crowing period of early maturity groups (P-P1), 62 d early crowing period of early maturity groups (P-P2), 32 d pre-crowing period of late maturity groups (L-P1) and 109 d early crowing period of late maturity groups (L-P2) had 10 samples respectively, were sequenced by Tag-DGE technique, and the bioinformatics methods were analyzed subsequently, such as sequencing assess and gene function annotation. The results showed that 8,760,215 reads and 422,828,562 bp data were obtained in P-P1, 10,330,840 reads and 497,528,131 bp data were obtained in P-P2, 14,830,588 reads and 717,240,559 bp data were obtained in L-P1, 11,097,283 reads and 536,297,125 bp data were obtained in L-P2. Respectively, 93.20% , 92.77% , 93.45% and 93.18% reads could be compared to the chicken reference genome sequence. As the pre-crowing period for reference, 22 differentially expressed genes were found in the early maturity groups and 27 differentially expressed genes were found in the late maturity groups. Through the GO/KEGG analysis of these different expression genes, six genes related with sexual precocity were selected out, which were LECT1, HAPLN1, GDFN, HTR1F, FSH β and OPN4.

Keywords: Ningdu Huang cocks, pituitary, Tag-DGE, differentially expressed genes

S2-0202 Chicken lines with different natural antibody levels differ in mortality to intratracheal aerial pathogenic *Escherichia coli* (APEC) infection at young age

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Natural antibodies (NAb) are antibodies present in individuals without known antigenic challenge. NAb levels binding keyhole limpet hemocyanin (KLH) in chickens were heritable and associated with survival suggesting that selective breeding may improve natural disease resistance. Four generations of a pure bred White leghorn line were divergently bred for total NAb levels binding KLH at 16 weeks of age, resulting in a high NAb line and a Low NAb line. Generation 4 (n_{high}=200, n_{low}=200) and control birds of the unselected parental line (n=200) were subjected to an intratracheal 108.2 CFU/mL *Escherichia coli* challenge in 0.2 mL PBS or to a 0.2 mL sham (PBS) challenge at 8 days of age. Mortality was recorded during 7 days post infection. Survivability was 100% for the sham challenged birds, regardless of line. Survivability was 91% for the high NAb line, 75% for the low NAb line, and 74% for the control line, respectively. These results suggest that breeding for enhanced NAb levels results in a lower mortality of layers to aerial pathogenic *E. coli* (APEC) infection at young age, but the mechanism(s) of protection remains to be studied. Measuring NAb levels can have great impact on current and future breeding and housing strategies for, and evaluation of disease resistance in poultry.

Keywords: natural antibody, breeding, disease resistance, *Escherichia coli*

S2- 0203 Real- time sexing of chick embryos and compatibility with in ovoprocedures

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The rapid and accurate sex identification of birds is desirable for a variety of research and industrial purposes but, unfortunately, traditional sexing techniques/methods are laborious and time consuming. There are a number of PCR-based molecular sexing assays available that use different sex-linked markers, and while these are relatively rapid, they are unsuitable for most industrial and in ovo procedures because of susceptibility to contamination and stringent time constraints. We have developed a real-time chick sexing assay using an isothermal, "PCR- free" approach. During assay development, every sample analysed (n>500) was also sexed using an established molecular sexing assay. In addition, this assay was used in 'blind' studies to analyse blood samples from 1000 adult birds. In all instances, the results of our assay were in total agreement with either the sex of the adult birds, or the sex of embryos as determined by established protocols. This sexing assay, is able to discriminate between male and female samples in less than 10 minutes using as little as 1 ng of genomic DNA, 0.125 ul of whole blood, or 250 cells. The use of this assay enables us to determine the sex of very early donor embryos (H&H Stage 12) prior to tissue transfer in live transplantation studies. In conclusion, we have developed a rapid, accurate and sensitive sexing assay that is compatible with in ovo manipulations.

Keywords: avian, sex, chick, embryo, sexing

S2-0204 Supplementing dietary sugar promotes endoplasmic reticulum stress-independent insulin resistance and fatty liver in goose

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It is known that endoplasmic reticulum stress (ERS) contributes to insulin resistance (IR) and non-alcoholic fatty liver disease (NAFLD) in mammals. However, we recently demonstrated that overfeeding with a traditional diet (mainly consisting of cooked maize) does not induce ERS in goose. As cellular studies show that high glucose and palmitate can trigger ERS in mammalian cells, we hypothesized that supplementing sugar to the traditional diet could induce ERS, thus promoting insulin resistance and fatty liver. To test the hypothesis, we first treated goose primary hepatocytes with high glucose (25 mM and 50 mM) and palmitate (0.5 mM) supplemented with or without 0.25 mM oleate. Quantitative PCR analysis indicated that, as in mammalian cells, high glucose and palmitate indeed induced ERS in goose primary hepatocytes, and palmitate-induced ERS was suppressed by supplemental 0.25 mM oleate. We then tested the hypothesis with an in vivo study, in which Landes geese overfed with traditional or novel diets (i.e., the traditional diet supplemented with 20% sugar in weight) were compared with control geese (normally fed with cooked maize) for ERS, IR and fatty liver. The differences in glucose tolerance and postprandial blood glucose between the geese overfed with traditional and novel diets suggested that supplementing dietary sugar promoted IR. This promotion was accompanied with an increasing trend of liver weight and abdominal fat weight relative to body weight. Surprisingly, compared to overfeeding with the traditional diet, overfeeding with the novel diet did not induce ERS, even further suppressed ERS in goose fatty liver. Together, our findings suggest that supplementing dietary sugar promotes ERS-independent IR and fatty liver in goose. It is intriguing to discover the factor(s) protecting goose liver from ERS as well as the non-ERS mechanism underlying IR.

Keywords: diet, endoplasmic reticulum stress, insulin resistance, non-alcoholic fatty liver disease, glucose tolerance test

S2-0205 MiRNA transcriptome analysis reveals the regulatory function of differentially expressed miRNAs in chicken hepatic lipid metabolism

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Laying egg is the most important economic trait to egg-laying hens, and this physiological process is highly dependent on the function of chicken liver. Increasing studies have evidenced that miRNAs serve as important regulators in hepatic metabolism. The poultry egg-laying process contains a great deal of lipid metabolic action, which could be highly associated with the hepatic miRNA-mediated biological process. To investigate the miRNA expression profile during lipid metabolism, miRNA-seq was adopted to the livers of juvenile and laying chickens. A total of 655 known miRNAs were obtained from juvenile and laying hens. Among them, 67 down- and 13 up-regulated miRNAs were verified to be significantly differentially expressed in laying hens. Integrated analysis of differentially expressed miRNAs and mRNAs showed that 648 putative target genes for the 80 significantly differentially expressed miRNAs were predicted. MiR-22-3p was showed interact with PLIN2 and ELOVL6, and miR-16-5p, miR-101-2-5p correspondingly interact with FASN and APOB. In vitro experiment, which chicken primary cultured hepatocyte was stimulated at a time course (0, 12, and 24h) by estrogen, showed that the expression of PLIN2, ELOVL6, FASN and APOB were significantly increased in a time-course pattern, while miR-22-3p, and miR-16-5p were significantly decreased. The expression level of miR-101-2-5p was the lowest, while the putative target gene APOB with the highest expression when treated for 12h. In addition, the bioinformatics analysis results showed that target genes significantly enriched in many lipid relevant biological processes. Differentially expressed miRNAs and their target genes in our study strongly suggest that they involve in chicken hepatic lipid metabolism.

Keywords: MiRNA, hepatocyte, chicken, estrogen, integrated analysis

S2-0207 Identification of candidate genes and molecular breeding markers for sperm storage capacity trait in YuKou Jing brown I hens

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The sperm storage capacity refer to time span of succession fertilized eggs after one natural mating or artificial insemination (AI) in hens (one infertile egg is allowed). The sperm store in sperm storage tubules (SST) which located in uterus-vaginal junction (UVJ) in oviduct. The capacity of sperm storage affect AI frequency, number of breeder roosters, labor cost and economic benefit of production. Thus it is an important reproductive trait. In this study, we conducted sperm storage capacity measurement using 984 YuKou Jing brown I parental hens. The results showed that there is significant variation among individuals. The average fertile rate was 93.66% and 86.43% in 5 and 10 days post-insemination in this population. The average fertile is 93.88% in 300 hens which have high storage capacity for 10 days. It reached 94.03% in 50 hens which owing ultrahigh sperm storage capacity for 15 days. Further, we investigated the molecular mechanisms that might resulted in significant different sperm storage capacity. A total of 1160 genes which expressed differently between high (>15 days) and low (<6 days) sperm storage capacity in UVJ tissues were identified by RNA-SEQ. GWAS analysis using the chicken 600k high-density SNP chip identified significant signal on chromosome 1 using 192 extreme YuKou Jing brown I parental hen individuals with high and low sperm storage capacity. Moreover, we found expression of TGFβ3 gene played an important role in hens storage capacity, indicating immunity response genes may play an important role in the regulation of sperm survival in SSTs.

Keywords: sperm storage capacity, YuKou Jing brown I hen, AI, SNP chip, TGFβ3

S2-0208 Expression profiles of novel genes and miRNAs involved in lipid metabolism in chickens adipocyte

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Excessive fat accretion is a crucial problem during broiler production. With the aim of identifying the novel miRNA and genes, the expression profiles of four candidate genes and miRNA involved in lipid metabolism were examined in undifferentiated and differentiated (3d, 5d, 7d and 10d) adipocyte by Q-PCR. Results showed that the expression of gga-miR30 cluster (gga-miR-30a, miR-30c-5p and miR-30e) in differentiated fat cell was significantly higher than that in undifferentiated state. Expression of miR-103-3p peaked after inducing differentiation 3d. As for the expression profiles of candidate genes: 1) Expression of acyl-CoA synthetase long-chain family member 1 (ACSL1), cytochrome b5 type A (CYB5A) and Sec23 homolog A (SEC23A) in undifferentiated cell was significantly higher than that in differentiated cell; 2) expression of brain protein 44-like (BRP44L) in undifferentiated cell was significantly higher than that in differentiated cell and was the lowest level after inducing differentiation 3d. From the analysis using the TargetScan and miRanda algorithms, BRP44L transcripts may be a target of miR-103-3p, and expression of miR-103-3p was negatively correlated with BRP44L. These findings suggest that four genes and miRNA are strong candidate genes involved in regulating the accumulation of lipid in chicken adipocyte; BRP44L may be an endogenous target of miR-103-3p, which need further identify by verifying of gene function and Luciferase reporter assay.

Keywords: chicken, adipocyte, miRNA

S2- 0209 The breeding of complete set line for dwarf hens

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Dwarf gene is feathered with short tibia and high feed conversion rate. The complete set lines of dwarf hens, CAU (China Agricultural University) No.3 and No.5 Layer were cultivated by breeding experts from BAU Animal Technology Co., Ltd. and CAU. For paternal line, total egg number, average egg weight and disease resistance are of main selected traits. For maternal line, target traits are mainly propagation, total egg number and weight. Independent elimination method is used for highly heritable traits. While, individual, family and combination selection are used for lowly heritable traits according to appearance, propagation and other important traits. In systematic breeding, a combination method of firstly selecting then expanding and firstly expanding then selecting is used. Through several generations' breeding, CAU No.3 Layer has a advantage of feathering auto-sexing. Its production abilities are as follows. Firstly, the survival rate is about 97.6% during the period of 0~18 week, and the it comes to 95.8% during the laying period. Secondly, the total number of egg-laying is 306 and the total weight of egg-laying is about 16.8 kg during the period of 19~72 week. Thirdly, the feed-egg ratio is about 1.92~2.00 to 1 during the laying period. The complete set line of CAU No.5 Layer not only makes parental generation feather color auto-sexing, but also gets commercial chick feathering auto-sexing. Meanwhile, it avoids the threat of virus since the female parent with endogenous viruses 21 from slow feather Leghorn. The advantages of commercial generation are as follows. Firstly, the egg color is pink and the feather color is red, which adapts to the breeding goals due to the market needs. Secondly, the total number of egg-laying is to 298 during the period of 19~72 week. Thirdly, the total weight of egg-laying is about 16.4 kg. Fourthly, the feed- egg ratio is about 2.04 to 1 during the laying period. At last but not least, it could increase the residual value.

Keywords: Dw gene, CAU No.3 layer, CAU No.5 layer, complete set line, auto-sexing

S2-0210 Evaluation of genetic diversity of Jingyang fowl

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Evaluation of genetic diversity of Jingyang fowl by using 5 Microsatellites Abstract Background: JingYang fowls, a native breed located at JianShi in Hubei province, are well known for its large body size, good meat quality, high nutritive value, as well as its well roughage-resistance. In 2009, JYF became one of the Geographical indication protection products, and the natural conservation areas were set up subsequently with the support of government and HZAU. Our research objective is to evaluate the genetic diversity of JYF, which is the basis of further study on genetic resource conservation. Materials and methods: A total of 94 individuals from 1 population were examined, and the total experiment contains several parts involving material acquisition, DNA extraction, PCR amplification, AGE, PAGE. Genomic DNA was extracted respectively from the blood in female and sperm in male. 5MS were investigated for the evaluation of Genetic diversity which were selected from the recommended MS by ISAG, after PCR amplification, we use AGE to confine the objective band and then use PAGE to separate them. Results and Conclusions: 1. A total of 95 alleles were detected from 5 MS markers in JYF, ranging from 13 to 25 alleles with average of 19 alleles per locus; 2. The obtained average PIC value per locus was ranged from 0.87 to 0.92, which indicates that Jingyang Chicken has rich genetic diversity, but most sites do not conform to the hardy-weinberg equilibrium, and in a state of heterozygote deficiency; 3. The sign test and WILCOXON test show that chicken population balance is not in conformity with the mutation - drift. Jingyang line allele L distribution results showed that the Jingyang chicken has not a recent genetic bottleneck.

Keywords: Jing Yang fowls, genetic diversity, microsatellites, population

S2-0211 Pattern of GHR mRNA expression and body growth in S2 line of sex-linked dwarf chicken

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The sex-linked dwarf chickens (SLD) have been widely used in the cross breeding of broilers and laying hens. With the aim of studying the molecular mechanisms underlying GHR in SLD chickens, the expression profiles of GHR were detected in three growth related tissues (liver, breast, thigh) in female and male of S2 line of SLD chickens at seven stages (d1, 3wk, 7wk, 9wk, 11wk, 13wk and 15wk) and growth curve of body weight was fitted by Logistica and Gompertz model. Results showed that the inflexion week and inflexion weight in male chickens was earlier than that of in female chickens. As for the expression profiles of GHR, there was no significant differences between tissues at hatching; the expression peaked at 7wk and dropped by degrees in muscle; hepatic expression increased with age and was positively correlated with body weight. Taken together, the present study would provide a theoretical basis for further study on the molecular mechanism underlying GHR regulation in SLD chickens.

Keywords: sex-linked dwarf chickens, GHR, growth curve

S2-0212 Mechanism research on the fresh egg content spreading area of White Leghorn chickens

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Egg content spreading area is a simple and important egg quality indicator when an egg was broken and the egg content was poured onto a horizontal plate. However, there are few reports about the egg content spreading area recently. Aimed to estimate the genetic parameters of egg content spreading area by DMUv6, 3102 eggs laid by 1034 layers at the age of 44 weeks of White Leghorn line were collected, 3 eggs per layer. We measured the total egg content spreading area(TECA), outer thin albumen area(OTAA), inner thick albumen area(ITAA) and yolk area(YA) by the means of digital image analysis and egg weight(EW) immediately after the eggs laid. In addition, 38 layers having extremely large TECA and 38 layers having extremely small TECA were selected. We collected 114 fresh eggs laid by these layers, 3 eggs per layer and measured the TECA, OTAA, ITAA, YA, EW, eggshell strength(ESS), eggshell thickness(EST), eggshell deformation(ESD) and the pH, weight and moisture content of both outer thin albumen and inner thick albumen. The results show that the heritability of TECA, OTAA, ITAA, YA and EW was 0.24, 0.21, 0.48, 0.26 and 0.36, respectively. The ITAA, YA, EW, ESS, EST, ESD and the pH and moisture content of both thin albumen and thick albumen have no remarkable difference($p>0.05$) between the layers selected based on TECA, while there have very significant difference($p<0.01$) in TECA, OTAA and the weight of both outer thin and inner thick albumen by T-test. In conclusion, TECA, OTAA, ITAA, YA and EW all have a moderate or high heritability. The outer thin albumen weight and inner thick albumen weight affect the egg content spreading area greatly. The reasons caused difference in egg content spreading area must be explored further.

Keywords: egg albumen area, genetic parameter, albumen pH

S2- 0213 Genetic diversity analysis of mtDNA D-loop region in Xichuan Black-bone chicken

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It was becoming more and more common through the mitochondrial DNA to explore the genetic diversity. To determine the origin and genetic diversity of Xichuan Black-Bone chicken, genomic DNAs extracted from blood samples of 50 individuals were used to analyze its hyper-variable region I sequence variations in mtDNA D-loop. The mtDNA D-loop sequences of 11 chicken breeds, including White Plymouth Rock, White Leghorn, Rhode Island Red, New Hampshire, G.g.gallus, G.g.spadiceus Yunnan, G.g.spadiceus Myanmar, Gushi Henan, Cockfight Henan, Luxi Shandong and Souguang Shandong download from GenBank database were used to analysis. For the Xichuan Black-Bone chicken, the mtDNA D-loop HVS-I sequence was amplified by using PCR and the PCR products were directly sequenced. At first, the raw sequence obtained were edited and aligned using the Edit sequence and DNASTAR. Haplotype diversity and nucleotide diversity were computed by using DNASP version 5.0. Secondly, the genetic distance was computed, and a neighbor-joining tree of all the haplotypes under the Kimura 2-parameter model was constructed by using MEGA 5.1 software. The results showed that 21 mutation sites and 10 haplotypes were found in mtDNA D-loop of Xichuan Black-bone chicken, haplotype diversity was 0.867 ± 0.022 , and nucleotide diversity was 0.00882 ± 0.00273 . The genetic distances were ranged from 0.0019 to 0.0235, and the average genetic distance of haplotypes was 0.0140 ± 0.0031 . All the samples in the neighbor-joining phylogenetic tree were clustered in 4 clades. Our results suggested that the genetic diversity was relatively rich, and there were 4 maternal origins existed in Xichuan Black-bone chicken population. The study results would be a reference for genetic resources conservation development and utilization of Xichuan Black-bone chicken.

Keywords: Xichuan Black-bone chicken, mtDNA, D-loop region, haplotype, genetic diversity

S2-0215 Regulatory mechanism of a novel lncRNA- lncLMR in Gallus gallus

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LncRNAs are a group of new type RNAs that possess the similar structure characteristics of mRNA, but don't encode proteins. To investigate the effects of lncRNAs on lipid metabolism of liver in Gallus Gallus, lncRNA-seq was employed to obtain the lncRNA expression profile of the liver of juveniles and laying hens. A novel lncRNA- lncLMR, which was differently expressed with a 8-fold increase between juveniles and laying hens, was singled out. To confirm the biological functions and regulation mechanism of the lncLMR, the lncLMR was cloned, its tissue distribution and expression profile during sex maturation were analyzed during Lushi green-egg-shell chicken as a model by real-time PCR. And the lncLMR was further investigated by using primary cultured chicken hepatocytes treated with 17β -Estradiol and agonist and antagonist of estrogen receptors. The results showed that the lncLMR with the highest level in liver. The expression levels of lncLMR in liver between the chickens at the age of 15 weeks old (0.30 ± 0.12) and those at the age of 20 weeks old (0.44 ± 0.24) and 30 weeks old (3.60 ± 0.79) were highly significantly different. The expression levels of lncLMR were increased when primary cultured chicken hepatocytes were treated with 17β -Estradiol, but no change was found when the hepatocytes were treated with GPR30 agonist and estrogen receptors α antagonists. Therefore we concluded that the lncLMR was highly expressed in liver, up-regulated by estrogen via estrogen receptor β , and could be involved in lipid metabolism in chicken liver.

Keywords: lncRNA, liver, estrogen, expression and regulation

S2- 0216 PDSS2 (– 103C>G) mutation causes silky-feather in Guangxi Donglan–Wu chicken

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Guangxi Donglan-Wu chicken is a native chicken in Guangxi province, which has two kinds of feather, one is silky-feather phenotype, another is wild-type contour feather. It is unclear on the molecular mechanism of silky-feather in Donglan-Wu chicken. In previous study showed that silky-feather phenotype was caused by the -103C>G mutation of prenyl (decaprenyl) diphosphate synthase, subunit 2 (PDSS2) in most of silky-feather chicken. Therefore, the current study is to investigate whether the PDSS2-103C>G mutation is the reason of silky-feather phenotype in Guangxi Donglan-Wu chicken. 40 blood samples of Guangxi silky-feather and wild-type contour feather Donglan-Wu chicken were collected and genomic DNA were isolated. Special primers were designed according to the chicken PDSS2 sequence released on GenBank. PCR products were sequenced. The results showed that there were the transversion from C to G in upstream 103 of PDSS2 only in 20 Donglan-Wu chickens with silky-feather, but not in wild-type contour feather. Our results further support the result of previous study and suggest that the molecular mechanism of silky-feather formation in Guangxi Donglan-Wu chicken is the same with other silky-feather chickens.

Keywords: Guangxi Donglan-Wu Chicken, silky-feather, PDSS2 mutation

S2- 0217 Expression and functional characteristics of melanocortin receptor (MCR) and its accessory protein (MRAP) in liver in gallus gallus

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G protein coupled receptor (GPCR) plays important roles in GPCR signal transduction. Many accessory proteins interact with GPCRs, and alter either the GPCRs' ligand binding mechanism or their functional responses. The melanocortin receptor (MCR) accessory proteins (MRAPs) are such GPCR accessory proteins that function in the melanocortin system. In mammals, MRAP and MC2R (one of MCR family members) are mainly expressed in adrenal gland, and interact directly to traffic the MC2R from endoplasmic reticulum to the cell surface, where it mediates the effects of pituitary hormone ACTH. In chicken, five MCR family members (MC1R, MC2R, MC3R, MC4R, MC5R) and two MRAPs (MRAP, MRAP2) were predicted in chicken genome. To gain insight into the biological functions of the genes, we cloned the MRAP and MRAP2 genes. Sequencing analysis revealed that the functional domains of MRAP and MRAP2 were conserved between species. It suggested that the physiological roles of chicken MRAP and MRAP2 could be similar to their mammalian counterparts. Tissue expression distribution demonstrated that MRAP was presented in liver, adrenal gland, spleen, glandular stomach, and lungs, while MRAP2 is predominantly expressed in adrenal gland. All of the five MCRs were presented in adrenal gland, but showed different expression patterns in other tissues. Since the MC5R was the only MCR member who expressed in chicken liver, we hypothesized that the MRAP might interact with MC5R to function in chicken liver as it did in adrenal gland in mammals. However, expression levels of MRAP in liver were significantly increased with sex maturation, but no changes of MC5R expression were found. Moreover the expression of MRAP was up-regulated when chicken primary hepatocytes were treated with 17 β -Estradiol, but no effect on MC5R was observed. We concluded that MRAP may play a role alone rather than functioning by forming MRAP/MC5R complex in liver of chicken.

Keywords: chicken, melanocortin receptor, melanocortin receptor accessory proteins

S2– 0218 Natural autoantibodies in healthy chickens, a potential tool for enhancing disease resistance?

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Genetic selection for enhanced innate immunity might be a useful tool to improve general disease resistance. Natural antibodies (NAb) perform important functions within innate immunity. NAb in layers have been shown to be heritable and predictive for survival. A proportion of NAb bind self-molecules, or slightly changed self-molecules (neo-epitopes), or antigens shared by self and microbial structures, labeled as natural autoantibodies (NAAb). These NAAb are likely involved in maintaining physiological and immune homeostasis by removing aging or tumor cells, or cellular debris. Quantitative and qualitative differences in NAAb binding autoantigens and changes in NAAb profiles over time can represent various physiological and immune conditions. The objective of our project was to explore the genetic background of NAAb in poultry. We used the data from a population divergently selected for high or low NAb levels. We estimated genetic parameters for IgM and IgG antibodies binding different autoantigens in plasma of healthy layers. The estimated heritabilities ranged from 0.10 to 0.17 for IgM, and 0.02 to 0.11 for IgG, respectively. For both IgM and IgG, high genetic correlations were observed between levels of NAAb binding different autoantigens. In addition, significant maternal environmental effects were observed for IgM. Currently, we perform a genome wide association study in order to identify genomic regions involved in regulating NAAb levels. This will provide more insight into the genetic basis of innate immunity and possibly find genes underlying variations in NAAb levels.

Keywords: chicken, natural autoantibodies, autoantigens, genetic analysis

S2– 0219 Preservation and usage of genetic resources in farm poultry

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In 2007 FAO has developed and approved GLOBAL PLAN OF ACTION FOR ANIMAL GENETIC RESOURCES and the INTERLAKEN DECLARATION which aimed to prevent an erosion of genetic diversity in farm animals, preserve genetic resources and efficiently use them for improvement of animal populations to increase production efficiency. World experience confirms, that loosing of the natural diversity results in elimination of unique genotypes, narrowing of genetic potential, that decreases a base of breeding process and creation of new strains and breeds in the future. All - Russian Research Institute for Farm Animal Genetics and Breeding (VNIIGRZh) more than 40 years has been carrying investigations and practical work for preservation of 46 chicken breeds and populations, both Russian and foreign ex situ and in situ. Most common criteria for evaluation and preservation of those breeds are: livability, adaptability, health status, reproductive traits, unique genetic polymorphism on morphological and molecular levels, including use of DNA-markers. Besides gene pool preservation also are being carried put investigations for its practical usage: 1) Use of local breeds for creation of commercial meat-type 3-strains hybrids for farmers 2) Creation of specialized populations of white shelled hens for production of embryos for biological industry (production of embryonic vaccines both for medicine and veterinary application) 3) Investigation of cryopreservation adaptability of cocks' sperm of various breeds for preservation of genetic resources ex situ. 4) Search of DNA-markers of commercially valuable traits. Theoretical and practical solution of the problem of preservation of local and rare breeds is connected with necessity of application of various scientific approaches- from molecular and population genetics up to zootechnical methods and traditional breeding.

Keywords: poultry, breeds, breeding, gene pool preservation

S2-0220 Effects of short-term divergent selection for thirty- five- day body weight on growth and reproductive traits of Japanese quail

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A study was conducted to determine the effects of direct and correlated responses to divergent selection for 35-day body weight on growth and some reproductive traits in Japanese quail. A total of 1,500 chicks obtained from the National Veterinary Research Institute, Vom, Nigeria served as the base population. Divergent selection of base population was carried out over a period of five generation. The High Line consisted of all individual birds having body weight greater than one standard deviation above the population mean weight while all individuals which had body weight less than one standard deviation below the population mean were designated as the Low Line. A random sample of the base population was maintained as the Control Line for correcting environmental fluctuations. Random mating was made within Line at the ratio of one male to three females and eggs were collected according to Line for incubation to propagate subsequent generations. Data obtained were analysed within and between Lines using the One-way Analysis of Variance procedure. Total selection responses after 5 generations were 29.75g for the High Line males and 52.90g for the High Line females. The High Line males were 93.80g larger than the Low Line males while the High Line females were also 99.61g larger than their Low Line female counterparts. The High body weight Line attained sexual maturity at a significantly ($P<0.05$) lower age (40.56 days) than the Low body weight Line (59.78 days). Body weight at sexual maturity was highest in the High body weight Line (143.63g). Mean percent fertility showed no significant differences ($P>0.05$) among Lines but was highest in the Low (86.07%). Mean percent hatchability of incubated eggs and fertile eggs were however highest in the High Line though differences were not significant ($P>0.05$). It was concluded from the study that selection for increased 35-day body weight can help improve growth traits and reproductive characteristics in the Japanese quail.

Keywords: high line, low line, control, body weight. Japanese quail

S2- 0221 Estimates of genetic and phenotypic correlations, and heritability, of egg quality and production traits in two lines BPR layers

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The eggs production and eggs quality parameters by laying hens are influenced by many factors. The objective of the present work was to estimate heritability of, and genetic and phenotypic correlations between egg quality traits: Egg shell strength, Eggshell deformation and Egg weight at 28 weeks, Eggs weight at 35 weeks , Eggs weight at 64 weeks and Egg production traits: Hatchability of eggs set , % Lay between 20-40 weeks , Body weight at 18 weeks , Body weight at 65 weeks, Laying persistency as number eggs in last moths of lay , in two lines BPR layers tested in individual cages technology. The three samples of eggs were evaluated for every hen in the age of 35 weeks Egg shell strength, Eggshell deformation and Egg weight between years 2011 and 2013 in three next generations. In analysis were included 2118 hens as with performance and 3189 animals in pedigree (5 generations). Genetic parameters were estimated using mutli-trait animal model through REML method. The statistical model included the fixed effects of line, year, hatchery and breed and random effects for permanent environmental and direct effect of animal and residual error. The most influential fixed effect was effect of year. The results are in Table 1. The results suggest that additive genetic variance and genetic correlations analysed traits can be exploited in specific breeding program. This research was funded by an "S" grant of the MEYS of the CR.

Keywords: BPR layers, egg shell quality, production parameters, heritability, genetic correlations, phenotypic correlation

S2-0222 Destabilizing effect of selection in chicken: factors of variability

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The goal was to find out in which extent “destabilizing effect of selection” reveals the intact, original variability in domestic poultry and how it can be used in practical selection. The investigations have been carried out on chicken populations “Russian White” (a) and “Black and white Australorp” (b) in which for a long time have been carried selection for reaction of day old chicks on low ambient temperature (a) or stimulation of ACTH secretion (b). Within 4-6 generations there were found and consolidated genotypes: resistant to the low ambient to or with high levels of functional reserves of adrenal glands (FRAG) at stimulation of ACTH. The concentration of corticosterone (FRAG) became increased in 1,7 times (up to 57 ng/ml) compared to the initial generation (P0). Breeding “in itself” of the new genotypes resulted in dramatic changes, related to the fluff color of day old chicks (it turned into white) (a) and feather color of adult birds (b). In Black and white Australorps it led to the new feather coloration - grey and blue and on this base there was established a new population “Aurora”. Average level of FRAG in this population was even higher - 91,7 ng/ml. Response on thermal stress or ACTH acted as destabilizing factors, which determined directions of divergence by the following traits: body weight, day weight gain, egg productivity, morphological composition of blood, adaptability content of immunoglobulins. Correlated changes took place also in hormonal activity of thyroid (T4) and sex glands (progesterone, testosterone). On the base of the population “Russian White” with white coloration of fluff in day old chicks there was successfully carried out selection for resistance against neoplasm pathogens. Termination of selection pressure revealed that even after 25 years those genotypes demonstrate the similar response on ACTH stimulation [ABBiochem Co., Ltd., Shanghai, China]. FRAG in “Aurora” population was 84,8 ng/ml, “Russian White” - 82,6 ng/ml.

Keywords: breeding, stress, ACTH, chickens

S2- 0223 Genetic parameter estimates for eight-week body weight in a foundation stock of male line broiler chickens

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Abstract The objectives of this study were to estimate heritabilities, phenotypic and genetic correlations for eight-week body weight in a foundation stock of male line broiler chickens after 3 generations of selection. A total of 2,086 birds comprising 1500 hens and 586 cockerels were used in 3 generations. At hatch, chicks used were wing-banded, weighed and housed on deep litter. Body weights of birds were subsequently taken at 2, 4, 6 and 8 weeks of age. Mating ratio was 1: 6 across families in all generations. Selection at 8 weeks of age, was done based on highest body weight at that age. Growth performance was studied within generations. For each generation, data obtained were utilized to estimate heritabilities, genetic and phenotypic correlations from sire component of variance. Selection action (Sel action) statistical software was used to estimate heritability, genetic and phenotypic correlations. Correlations were moderate to high. Heritability estimates for generation 1 ranged from moderate to high 0.34 (BWT2) to 0.75 (BWT8), low to high for generation 2 (0.17) for BWT0 and (0.88) for BWT8 and positively low for BWT0 to high for BWT8 (0.16 to 0.81) for generation 3. This points to the existence of an appreciable amount of additive genetic variance in the flock and indicates that improvement in the traits can be brought about by intrapopulation selection. The high and positive genetic and phenotypic correlations indicate the pleiotropic action of genes controlling these traits, and that by direct selection for anyone of them; genetic improvement in the others will be realized as correlated responses.

Keywords: broiler, male line, foundation stock, heritability, correlation

S2- 0224 Inhibition of C122814 lncRNA in goose fatty liver and regulation by non-alcoholic fatty liver disease associated factors

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Long non-coding RNAs (lncRNAs), a key player in gene regulation, are involved in the occurrence of several diseases. However, the role of lncRNAs in goose fatty liver is unclear. To build a basis for addressing this, we first screened for lncRNAs that were differentially expressed in the livers of the overfed (n=3) versus normally-fed geese (n=3) using RNA-seq technology. A total of 2599 lncRNAs were identified in goose liver with 212 upregulated and 140 downregulated lncRNAs in fatty liver vs. normal liver. Among these differentially expressed genes, the expression of C122814lncRNA was validated by quantitative PCR (n=6). This lncRNA was significantly inhibited by 19-day overfeeding. To understand the regulation of its expression, we treated goose primary hepatocytes with glucose and insulin at different doses (n=3) as hyperglycemia and hyperinsulinemia were often associated with non-alcoholic fatty liver disease. Quantitative PCR analysis indicated that the expression of C122814lncRNA was upregulated by glucose/insulin, respectively. To test if the upregulation C122814 lncRNA by glucose in the cell study could be recapitulated in vivo, we overfed Landes geese with normal diet (mainly consisting of cooked maize, 1% oil and 1% salt) and sugar-supplemented normal diet (20% sugar in weight supplemented to the normal diet) for 19 days (n=4). Compared to the geese overfed with normal diet, the expression of C122814lncRNA was further decreased by ~22% in the livers of the geese overfed with sugar-supplemented normal diet. This was conflicted with the in vitro finding, suggesting other unknown factors play a role in the regulation of C122814lncRNA during the development of goose fatty liver. In conclusion, a total of 352 lncRNAs were differentially expressed in the livers of the overfed vs. normally-fed geese, and C122814lncRNA could be regulated by glucose in vitro and vivo. The mechanism underlying the regulation of the gene warrants further investigation.

Keywords: lncRNA, goose, non-alcoholic fatty liver disease, hyperglycemia, hyperinsulinemia

S2- 0225 Host and virus transcriptome response after ALV injection in SPF chicken

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Avian leukemia virus (ALV) is detrimental to poultry health, causing substantial economic losses from death and decreased performance due to tumors or immune system damage induced by ALV. No effective vaccine exists and the etiology of ALV remains poorly understood. Because tumorigenesis networks are very complex, the regulatory architecture of the immune system may include added dimensions of modulation by epigenetic miRNAs and lncRNA. To explore ALV damage, host immune resistance and the host-pathogen interaction, we developed a novel methodology that combines significant evidence of mRNA expression association with their regulator factors of miRNA and long-noncoding RNA expression, and interactor ALV virus expression. Special pathogen-free (SPF) layer hens were separated into 2 groups on 1d: ALV-J that were intraperitoneal infected and control. Spleen samples (3 from each group) were harvested at 40dpi and sequenced. Between the infected and non-infected group, 1,664 genes, 7 miRNA, 14 lncRNA exhibited significantly differential expression. The combined power of the triple RNA approach revealed that ALV infection affects many previously unreported canonical pathways and biological functions, including retinoic acid mediated apoptosis signaling, Death receptor signaling, UVA-Induced MAPK signaling. Eight canonical pathways were significantly activated/inhibited and shared among all 4 RNA types. IRF1, IRF7, and IFN were identified as the upstream factors during the ALV infection. This experiment identified transcript markers that may be indicators of disease, as well as identifying potential pathways and functions affected by the disease. Additionally, integration of information from the host transcriptomic and virus response has potential to provide deeper insights into other host-pathogen interactions.

Keywords: ALV, RNAseq, miRNA, lncRNA, spleen, chicken

S2- 0226 Mapping genetic loci for the maternal behaviour of incubation in chickens

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Introduction: The separation of maternal care and reproduction has helped poultry to become the major source of animal protein. Artificial incubation was perfected 3000 years ago in Egypt and China, replacing natural incubation and removing restrictions to production. It became possible to breed chickens with persistent egg production and no incubation behaviour, e.g. White Leghorn (WL). Conversely strains like the Silkie (SLK) are prized for their incubation behaviour, often known as broodiness. **Aim:** To discover the genetic loci associated with the loss of maternal behaviour and increased egg production of strains such as the WL. **Methods:** An F2 cross between WL and SLK breeds was genotyped with 340 markers. Phenotype was recorded daily on 280 hens housed between 16 and 52 weeks of age. Pens contained nest boxes with eggs to encourage incubation behaviour. Two phenotypes were used i) incubation status on a 3-point scale from full to no incubation; ii) early incubation behaviour from 25 and 30 weeks of age on a 2-point scale from no to full incubation behaviour. QTL analysis was carried out using GridQTL. **Results:** The founder WL and SLK hens showed 0 % and 90.5 %, the F1 97% and the F2 hens 46 % incubation behaviour. A genome wide significant QTL on chromosome 8 (21cM; F 11.8) for early incubation behaviour explained 12% of the difference between founders and a suggestive QTL on chromosome 1 (70cM; F, 5.3) for incubation status explained 26% of the difference between founders. **Conclusions:** We have mapped, for the first time, genetic loci that affect maternal behaviour and therefore differences in egg production. The QTL on chromosome 8 and 1 are most likely to contain genes that directly influence incubation behaviour because the SLK allele promoted incubation behaviour. The coincidence of a number of the QTL with genes of the thyrotrophic axis suggests an avenue to investigate regarding the loss of incubation behaviour.

Keywords: genetics, broodiness, reproduction, QTL

S2- 0227 Review on “Breeding for 500 eggs in 100 weeks” in layer

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Extending the laying cycle in commercial layer can reduce the cost of replacement pullets. Age of spent hen generally extend to 80 weeks now from 72 weeks past. In 2011, “Breeding for 500 eggs in 100 weeks” was proposed in 2010 and expectedly realized in 2020 by Institut de Sélection Animale (ISA). The goal is to breed layer that produce 500 eggs in 100 weeks of age, without molting. In an experiment of the ISA, there were already pure line birds that produce 500 eggs by 100 weeks of age, although the frequency was still relatively low. The record of a hen with the highest egg number was 571. We collected the latest data of several commercial layer breeds (ISA-White, Hy-Line W-36, ISA-Brown, and Hy-Line Brown) from web sites of the companies (www.isapoultry.com and www.hyline.com). The results showed that in the optimum conditions laying rate at 80 weeks of age was 80% and at 90 weeks was 70%. So, even though 80% could retain to 90 weeks and 70% could retain to 100 weeks, the last 20 weeks only could produce 105 eggs. Along with eggs yield before 80 weeks of age, the total egg number only reached 480 by 100 weeks. Furthermore, MCM (modified compartmental model) equation that could perfectly fit laying rate curves of hen-day and hen housed layers from the 21st week of age to the 72nd week of age was used to fit curves of laying rate and obtained curves of “prediction 1” and “prediction 2” for “500 eggs in 100 weeks” through parameters adjustment. The results showed that the laying rate of “prediction 1” curve should remain above 84.3% at the 80th weeks, above 82.2% at the 90th weeks and above 80.2% at the 100th week, and the laying rate at peak period of “prediction 2” curve was 105.7%, which have some indexes that were difficult to achieve in the both predicted curves. So, the “500 eggs in 100 weeks” plan is almost impossible realized in large-scale commercial herds in 2020. Even if the goal of “500 eggs in 100 weeks” will not be realized, it still can be considered as a reference to layer breeding programs. Breeding companies in China may introduce mature synthetic lines from abroad or breed own new breeds / synthetic lines with introducing genetic materials.

Keywords: layer chicken, breeding program, 500 eggs in 100 weeks

SS2-0228 Dwarf chicken with lower phagocytosis product show higher performance

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Monocytes are centrally involved in both specific and nonspecific immunity by secretion of regulatory immune mediators, phagocytosis and presentation of antigens. Previous studies have found that phagocytosis of peripheral blood monocyte can effectively reflect the disease resistant potential of poultry. The current study investigated the effect of selection for monocytes-phagocytosis on disease resistance in generation 0 (G0) of dwarf chickens. Eight hundred and ninety dwarf chickens were divided into high and low Phagocytosis product (PP) groups (HPPG and LPPG) based on PP of monocytes at 12 wk of age. Serum hemagglutination inhibition antibody titers were examined after inoculations of avian influenza virus H9 inactivated vaccine (57 wk) to study the relationship between PP and immune response. The results show that selection for HPPG benefited to humoral immunity in dwarf chickens ($P < 0.05$). The weight (0wk, 6wk, 18wk), shank length (6wk, 18wk), laying rate and mean egg weight from 20 to 24wk were analyzed. To gain insight into effects of selection for PP on fertilization rate, hatchability and healthy chick rate, 2×2 mating combinations were conducted. There were effects of selection for LPPG in hens on laying rate of 24wk ($P < 0.05$), mean egg weight of 23wk and 24wk ($P < 0.05$), fertilization rate ($P < 0.01$), hatchability ($P < 0.05$). However, hens' PP had no effects on weight (0wk, 6wk, 18wk), shank length (6wk, 18wk) or healthy chick rate. The results indicated that phagocytosis of peripheral blood monocytes might be an indicator of humoral immunity and suggest that disease resistance was negatively correlated with production traits in dwarf chicken.

Keywords: monocyte, phagocytosis product, production trait, dwarf chicken

S3-0003 A survey on the prevalence of *Campylobacter* in chickens in different farming practices in Papua New Guinea

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Campylobacter is one of the important causes of food borne diseases in human. Though food animals are the sources of infection, poultry is the major source. Though *Campylobacter* is associated with enteritis in some parts of PNG, the source of infection is not clear as there is no proper surveillance in place. Hence a study to know the prevalence of *Campylobacter* in chickens in four different regions of PNG was undertaken. Cloacal swabs were collected from commercial, semi-commercial and non-commercial poultry farms and subjected to real time PCR to assess the prevalence. Of the 413 samples from chicken, 121 samples were positive for *Campylobacter* (36.3%). In the southern highlands *Campylobacter* was present in 83.3% (5/6) of commercial farms and 6.1% (2/33) of non-commercial farm but were absent in semi-commercial farms (0/8). In NGI region prevalence of *Campylobacter* was observed at 50% (1/2), 29.4% (5/17) and 14% (8/57) in commercial, semi-commercial and non-commercial farm respectively. In Momose region, semi-commercial farms had the highest prevalence of *Campylobacter* at 57.9% (45/78), with commercial farms at 52% (13/25) and non-commercial farms at 12.7% (6/47). In Highlands *Campylobacter* was found to have a higher prevalence rate in semi-commercial farms at 57.4% (35/61) compared to commercial farms at 25% (26/104) and non-commercial farms at 17.4% (8/46). Lack of proper biosecurity measures in commercial and semi-commercial farms where as rearing other livestock along with chicken in non-commercial farms could be the reason for the prevalence of *Campylobacter*.
Keywords: *Campylobacter*, Papua New Guinea, chicken

S3-0006 Molecular and phylogenetic analysis of oncogenes from virulent serotype I Marek's disease viruses from Andhra Pradesh, India

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Marek's disease is a neoplastic and lymphoproliferative disease of chickens caused by an alphaherpes virus characterized by the presence of lymphomas in different visceral organs. In the present study blood and tissue samples were collected from different MD suspected outbreaks of poultry farms from Andhra Pradesh, India. The gross lesions and histopathology of the tissues were typical of the oncogenic serotype-I MDV's. From the suspected samples, DNA was extracted and subjected to PCR targeting a 132 bp tandem repeat region specific for serotype - I MDV's. Out of 27 blood samples 20 were positive and all the 84 tissue samples collected from MD suspected lymphomas were positive in PCR. From the positive samples, amplification of Meq and IL-8 genes was carried out using specific primers. The nucleotide sequence analysis and the phylogenetic analysis of the samples using MEGA 6.0 software showed that these viruses are 99.6 and 99.5 % homologous to RB-1B (very virulent) and GA (virulent) strains of MDV. The mutations in the Meq gene of MDV strains were found to be more significant in MD viral pathogenecity and oncogenecity.

Keywords: Marek's disease virus, PCR, phylogeny, oncogenes, India

S3-0007 Studying the efficacy of certain organic acids on zootechnical performance and colonization of Salmonella in infected chickens

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This work was designed to study the effect of using sodium butyrate encapsulated in palm fat when compared with enrofloxacin on a disease picture, zootechnical performance variables and intestinal colonization of Salmonella Enteritidis (SE) experimentally infected broiler chickens. Two hundred and fifty five, day-old broiler chicks were kept for 5 weeks. At arrival, 5 sacrificed chicks were cultured to confirm their freedom of Salmonellae. Chicks were randomly allocated into 5 equal groups (1-5), consisting of 50 birds each. Group (1) was kept without challenge or treatment (blank control), group (2) was fed on sodium butyrate, group (3) was challenged and treated with enrofloxacin, group (4) was challenged and treated with sodium butyrate, while group (5) was only challenged (positive control). Each chick in the challenged groups was orally inoculated with 0.3 ml (1.5×10^8 SE/ml) at the 2nd day of age. Enrofloxacin was given at the 3rd day of age in water (10 mg/kg bwt) for 5 successive days; however sodium butyrate was added for ration in doses of 1, 0.5 and 0.25 kg/ton for starter, grower and finisher ration respectively from day old till the end of study. The results revealed no mortalities and decrease in the severity of signs and lesions in the treated groups than positive control one. At the 4th week of age, sodium butyrate supplement gave a significant ($P \leq 0.05$) improvement in body weight, weight gain and feed conversion than others. The re-isolation rate and enumeration of (SE) were lower in sodium butyrate and enrofloxacin treatments than positive control. It could be concluded that, sodium butyrate as an acidifier could be used as an environmentally friendly supplement when compared with enrofloxacin for treatment of (SE) infection in broiler chickens as it could reduce the disease picture severity, improve performance variables and decrease the intestinal colonization.

Keywords: sodium butyrate, Salmonella, chickens, performance

S3- 0008 Co- infection of Newcastle disease virus with Low Pathogenic Avian Influenza virus-H9N2 exacerbates the clinical disease in Newcastle disease vaccinated layer flocks

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Genotype XIII Newcastle disease (ND) is increasingly reported in vaccinated poultry flocks of Asia. It also combined with Low Pathogenic Avian Influenza (LPAI) -H9N2 leading to severe disease. The disease pattern during co-infections of ND and LPAI is not well understood. Our study describes clinico-pathological patterns of co-infection of ND and LPAI-H9N2, which could further helpful to understand the interactions of these two viruses at field level. The study was carried out in 37 ND vaccinated commercial poultry flocks of India showing outbreaks during January 2010 - 2012. Necropsy examination was carried out, clinical samples were collected for microbiological and pathological studies followed by molecular diagnosis. Out of 37 flocks screened, ND virus (NDV) of virulent pathotype (class II, genotype XIII) was detected in 22 farms (59.5%) and LPAI virus H9N2 subtype in 11 farms (29.7%) by virus isolation, RT-PCR and sequencing. All the LPAI positive flocks were co-infected with NDV. The mortality was comparatively high in the flocks co-infected with NDV and LPAI (2.6~44.40%) than NDV alone infected flocks (0.8~12%). Flocks infected with only the NDV found to recover as early within 10-15 days, whereas the recovery period was prolonged to one month in co-infected flocks. Further, *E. coli* and mycoplasma were also detected from the co-infected flocks during progression of the clinical disease. The NDV alone as well as LPAI and NDV co-infected flocks exhibited clinical signs and lesions similar to that of virulent NDV except the degree of severity which was high in co-infected flocks. In conclusion, genotype XIII NDV cause disease even in vaccinated poultry flocks of India. The clinical disease and mortality with genotype XIII NDV were exacerbated by LPAI-H9N2 co-infection. Added to this, secondary bacterial infections with mycoplasma and *E. coli* also caused exacerbation of clinical disease leading to huge mortality.

Keywords: Clinico- pathological patterns, LPAI, NDV, co-infection

S3- 0009 Characterization of haemagglutinin and neuraminidase genes of H9N2 low pathogenicity avian influenza viruses from Southern India

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In India, the Low pathogenic avian influenza (LPAI) H9N2 subtype was first detected in 2003, since then it has been isolated from 10 more northern and eastern states. Systematical surveillance for the evolution of LPAI viruses in poultry flocks of Southern India is not available, where the poultry population accounts for more than 50% of the country's total poultry. In this context, the present study reports the isolation and molecular characterization of H9N2 LPAI viruses from the poultry flocks of the Southern India. A study was carried out in 30 poultry flocks with a history of respiratory complications, and production drops during January 2010 to 2012. Clinical samples were collected for virus isolation and molecular detection by PCR targeting M gene, followed by HA and NA gens and sequencing. Out of 30 poultry flocks screened, six were found positive for LPAI. Sequence analysis of the HA gene cleavage site revealed that all were belonging to H9N2 LPAI viruses (RSSR*G in HA cleavage site) and shared 100% identity with each other. Phylogenetic analyses of the partial nucleotides of the HA and NA genes revealed that multiple H9N2 genetic lineages have circulated in the poultry population of South Asia and in the Mideast during the last decade. Although the H9N2 subtype have been isolated from chicken and ducks in Eastern Asia, analysis of the HA gene suggests that it is different from the one documented in South Asia. Nucleotide sequences obtained from the viruses isolated in this study were genetically related and formed independent genetic lineages. Based on the HA gene, these viruses may be closely related to AIVs that were isolated in India between 2003 and 2006, and also in Bangladesh, although low statistical support for internal nodes in the phylogenetic tree preclude definitive conclusion on their origin. The analysis performed on the NA gene did not provided more precise information on the geographic origin of the viruses isolated in the present study.

Keywords: LPAI, H9N2, haemagglutinin, neuraminidase, characterization, India

S3- 0010 A conserved epitope mapped with a monoclonal antibody against the VP3 protein of goose parvovirus by using peptide screening and phage display approaches

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Waterfowl parvovirus (WPV) infection causes high mortality and morbidity in both geese (*Anser anser*) and Muscovy ducks (*Cairina moschata*), resulting in significant losses to the waterfowl industries. The VP3 protein of WPV is a major structural protein that induces neutralizing antibodies in the waterfowl. However, B-cell epitopes on the VP3 protein of WPV have not been characterized. **Methods and Results** To understand the antigenic determinants of the VP3 protein, we used the monoclonal antibody (mAb) 4A6 to screen a set of eight partially expressed overlapping peptides spanning VP3. Using western blotting and an enzyme-linked immunosorbent assay (ELISA), we localized the VP3 epitope between amino acids (aa) 57 and 112. To identify the essential epitope residues, a phage library displaying 12-mer random peptides was screened with mAb 4A6. Phage clone peptides displayed a consensus sequence of YxRFHxH that mimicked the sequence 82Y/FNRFHCH88, which corresponded to amino acid residues 82 to 88 of VP3 protein of WPVs. mAb 4A6 binding to biotinylated fragments corresponding to amino acid residues 82 to 88 of the VP3 protein verified that the 82F_xRFH_xH88 was the VP3 epitope and that amino acids 82F is necessary to retain maximal binding to mAb 4A6. Parvovirus-positive goose and duck sera reacted with the epitope peptide by dot blotting assay, revealing the importance of these amino acids of the epitope in antibody-epitope binding reactivity. We identified the motif FxRFHxH as a VP3-specific B-cell epitope that is recognized by the neutralizing mAb 4A6. This finding might be valuable in understanding of the antigenic topology of VP3 of WPV.

Keywords: waterfowl parvovirus, VP3 protein, monoclonal antibody, epitope, peptide screening and phage display

S3- 0011 Oligodeoxynucleotides containing CpG motifs (CpG- ODN) as an alternative to antibiotics in the poultry industry

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Oligodeoxynucleotides (ODN) containing CpG motifs (CpG-ODN) have been shown to stimulate the innate immune system against a variety of bacterial, viral and protozoan infections in a variety of vertebrate species including poultry. We have previously shown that in ovo delivery of unformulated CpG-ODN was able to significantly protect neonatal broiler chickens against *Escherichia coli* or *Salmonella typhimurium* infections. The objectives of this study were to examine the safety and immunoprotective effects of CpG-ODN formulated with 2 types of lipid-surfactant (LSC) delivery systems in neonatal broilers against *E. coli* septicemia. Embryonated eggs which had been incubated for 18 days, received either 50 µg of 50 µg of LSC-CpG-ODN, 50 µg of unformulated CpG-ODN or saline. Four days after exposure to CpG-ODN, 1×10^4 or 1×10^5 colony-forming units (cfu) of a virulent *E. coli* isolated was inoculated subcutaneously in the neck. Clinical signs, pathology, bacterial isolations from the air sacs, and mortality were observed for 8 days following challenge with *E. coli*. Bacterial isolations and pathological observations were conducted immediately after birds were dead or euthanized. The survival rate of birds in groups receiving saline following *E. coli* infection was 20% to 30%. In contrast, birds receiving CpG-ODN formulations had a significantly higher survival rate of 60% to 80% ($p < 0.01$). Bacterial loads and clinical scores were significantly lower ($p < 0.05$) in groups treated with LSC-CpG-ODN compared to the groups receiving CpG-ODN or saline. This study demonstrates the utility of CpG-ODN as an alternative to antibiotics in the poultry industry.

Keywords: immunostimulation, alternatives to antibiotics, poultry

S3- 0012 Molecular and histopathological determination of *Cryptosporidium* spp. in chickens in Iran

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Cryptosporidiosis is a parasitic protozoan disease of the phylum Apicomplexa that is regarded as one of the most prevalent infections in more than 30 species of domestic, wild, cage, and pet birds. Chicken, quails, ducks, turkeys, geese, pheasant, as well as ostriches and swans, are among the species that can be affected. Documented data shows three species of cryptosporidium as the cause of Cryptosporidiosis in poultry hosts which includes *Cryptosporidium baileyi*, *C. meleagridis* and *C.galli*. This study was undertaken to determination of cryptosporidiosis by molecular and pathological methods in industrial and native broilers in Guilan Province (North of Iran). One-hundred fecal samples and tissue samples from pleura, trachea and intestine of chickens in Guilan province were collected in 2014. Smears from both fecal and tissue samples were stained by modified Ziehl - Neelsen method. Molecular detection of *Cryptosporidium* in oocysts which recovered from fecal samples was processed by using Nested-PCR with 18srRNA locus gene. Results: From the examined chickens 8% were positive for *Cryptosporidium*. In microscopic study lesions of enterocyte detachment, small-intestinal hyperemia and villus atrophy, crypt hypertrophy, microvillus atrophy or hypoplasia, purulent inflammation was seen in alimentary organs. Hyperemia in trachea and epithelial cell metaplasia and hyperplasia were seen in respiratory organs. In this study low rate of cryptosporidiosis was determined in north of Iran. Due to high sensitivity of molecular procedures in determination of cryptosporidiosis, this method is recommended for diagnosis of the disease. For diagnosing this disease by pathologic aspect, it is considered that pathognomic symptoms are seen in alimentary and respiratory tracts.

Keywords: *Cryptosporidium*, determination, chicken, PCR

S3- 0013 Lactulose, rhamnose and mannitol sugars test to assess increased intestinal permeability induced by lipopolysaccharide in chickens

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Increased intestinal permeability (IP) can lead to bacterial translocation and compromised health. Limited in vivo IP research has been conducted in chickens. The objectives of this study were to develop a model of increased IP utilizing lipopolysaccharide (LPS *E. coli* O55:B5) and to evaluate IP changes by the lactulose, mannitol and rhamnose (LMR) sugar test detected by HPIC. Ross male chicks were raised on the floor until day 14 when they were transferred to individual cages in three separate experiments. Birds (n=36) were allocated to control and LPS-treated groups (n=18) for experiments 1 and 2. Each group was further allocated into subgroups of six (0, 30, 60, 90, 120 and 180 minutes) and three (60, 90 and 120 minutes) time points in experiments 1 and 2, respectively. Birds (n=32) were allocated to control and LPS (n=16) for one time point (90 minutes) in experiment 3. LPS was injected at doses of 0.5, 1 and 1 mg/kg body weight intraperitoneally in experiments 1, 2 and 3 respectively on day 16, 18 and 20 while controls received sterile saline. On day 21, only birds in experiments 1 and 2 were fasted for 19.5 hours. LMR sugars (0.25g L, 0.05g M&R per bird) were orally gavaged and blood collected once per bird after gavage as per time point. D- lactate and diamine oxidase (DAO) were also tested in experiment 3 (n =10) by ELISA. LMR sugar results were not different statistically ($P>0.05$) in the first two experiments. The L/R ratio was significantly different between treatments in experiment 3. Nevertheless, due to similar lactulose absorption ($P>0.05$) between treatments it was concluded that LPS treatment did not increase IP. This was also confirmed by DAO and D-lactate tests in experiment 3 ($P>0.05$). We conclude that LPS at doses of 0.5~1 mg/kg did not increase IP in this model system. However the LMR sugar test has potential as a new biomarker to evaluate IP changes in chickens, further identifying the 90 minute time point as the optimal sampling time.

Keywords: lactulose/rhamnose, lactulose/mannitol, diamine oxidase

S3- 0014 Reduction of Clostridium perfringens induced necrotic enteritis by a standardized blend of plant alkaloids in broiler chickens

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Two studies were conducted to evaluate the effect of a standardized blend of plant-derived isoquinoline alkaloids (IQ, Sangrovit® G Premix) in broiler chickens infected with *Clostridium perfringens* (C.p.). On day 0, in both studies, 2,250 day-old male Ross 708 broilers were treated with a coccidiosis vaccine at twice the normal recommended dosage to induce an *Eimeria* spp. infection. On day 18, 19, and 20, C.p. was added to the complete feed at a dose of 1×10^8 CFU/ml/bird (all birds, except treatment 1). Birds were randomly allotted to five treatments (n=9): 1) Negative Control (NC) - no additive, no C.p.; 2) Positive Control (PC) - no additive, infected; 3) IQ low (60 g/t), infected; 4) IQ high (120 g/t), infected; 5) BMD 50 (454 g/t), infected. Birds were kept in floor pens and had ad libitum access to water and feed. In Experiment 1, fresh litter was used at the start of the trial. The litter was not amended or replaced during the study. In Experiment 2, the litter from Experiment 1 was reused. In both experiments, inclusion of IQ increased ($p \leq 0.05$) body weight. Compared to the PC, FCR was improved significantly ($p \leq 0.05$) if IQ was applied at a dosage of 120 g/t feed in both experiments. No differences ($p > 0.05$) between IQ and BMD were observed for performance parameters. In both studies, low lesion scores were observed, indicating a subclinical Necrotic Enteritis. NE Mortality was reduced significantly ($p \leq 0.05$) in both experiments in birds fed IQ. IQ worked in a dose-dependent way. In conclusion, the inclusion of a standardized formulation of IQ supported animals in cases of C.p.-induced Necrotic Enteritis and stabilized performance. It proved to be as effective as BMD and therefore is a valuable tool in broiler production.

Keywords: necrotic enteritis, *Clostridium perfringens*, isoquinoline alkaloids, feed additive, phytonics

S3-0015 Caffeine changes blood biochemistry, hematology and enzyme activity in avian model

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Animals are used as models for studying human biology and disease, and as test subjects for the development and testing of drugs, vaccines, antibodies and hormones. Caffeine is one of the most widely consumed pharmacologically substance in the world, widely found in coffee, tea, soft drinks and chocolates. Since the effects of caffeine on human health and metabolic disease have been the focus of much debate; this trial conducted to assess the effects of caffeine on laying quail performance, blood biochemistry and hematological changes as an avian model. Fifty six, 20-week-old laying Japanese quails (*Coturnix coturnix japonica*) were randomly divided into two groups (28 birds each with 7 replicates): a control, which remained on a normal diet (no added caffeine), and a caffeine group, which was placed on a 15 mg/kg body weight/day caffeine-added into water. On d 21 of experiment whole blood samples were collected by heart puncture from 14 birds per group. Then, birds were killed and organs weighed. A t-test using the GLM procedure of SAS software (SAS, 2004) was used to estimate significant differences ($P < 0.05$). Results have shown that body weight, Moreover the levels of total protein, liver and spleen relative weight were not affected by caffeine ($P > 0.05$). Albumin, globulin, glucose, triglyceride, cholesterol and low density lipoprotein were remarkably higher in caffeine group vs control ($P < 0.001$). Hematocrit and hemoglobin also significantly increased in caffeine group ($P < 0.001$). Also alkaline phosphatase and lactate dehydrogenase activity were highly increased by caffeine supplementation ($P < 0.001$). Globally, in consist of many reports in human research; caffeine had crucial changes on quail blood parameters in current study. So this economical, easy handling bird could be considered as an appropriate substitute animal model to study the nutritional effect of caffeine on human and helping to investigate the etiology of metabolic disorders in future.

Keywords: avian models, blood biochemistry, caffeine, quail

S3-0017 Infections prevention

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Over the last years, interesting research models have been developed in Georgia to study impaired gut health in the absence of growth promoters. Coccidiosis is a disease that is caused by protozoan parasites of the genus *Eimeria*, developing within the intestine of most domestic and wild animals and birds. Seven species of *Eimeria* (*E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. tenella*) are recognized as infecting chickens. Although coccidiosis is a disease known for many years, it is still considered as the most economical important parasitic condition affecting poultry production worldwide. For the control of coccidiosis in chickens and turkeys, a number of preventive medications have been approved for use world-wide, but reduced sensitivity and resistance are increasingly important as no new anticoccidial compounds are known to be under development. Also live attenuated and non-attenuated vaccines are available, but next to cost reasons, the fact that live vaccines need host cells to replicate and to instigate an active immunity, cause them to result in subclinical coccidiosis and this is a disadvantage. This is associated with a diminution of performance and, in the absence of growth promoters, even attenuated vaccines are considered by many poultry producers to be associated with a higher incidence of bacterial enteritis. In spite of this, live vaccines are significant and important tools in the anticoccidial arsenal, as will be explained in this paper.

Keywords: infections, diseases, coccidiosis, poultry

S3-0018 The report of severe cnemidocoptiasis and its pathological lesions responsible for the death of Budgerigars (*Melopsittacus undulatus*) in Iran

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Four dead Budgerigars (*Melopsittacus undulatus*) with lesions on face and legs were referred to animal clinic of veterinary collage in university of Tabriz in Iran. In the face, there was a keratin-like mass with the dimensions 2 to 10 mm in the lateral corner of left lower eyelid. Beak's size had not increased. The lesions in the face started from the corner of the beak and continued to base of the beak and the regions around. In legs, whitish, scaly lesions were observed. The lesions were mostly bilateral in legs and were also observed in dorsal and plantar regions of fingers (tarsal and metatarsal areas), which was described as thickened scales and hypertrophic crusts. The scrape was collected and preserved in 70% ethanol. The mites were isolated from scrape, cleaned and mounted directly. Specimens were incubated and the rounded cover glasses were sealed by nail enamel. In this study, tentative diagnosis of scaly leg infestation was based on character of lesion, affected host organs and species of hosts. However, the actual diagnosis was confirmed by laboratory identification of the causative mites under compound microscope. According to the lesions and by comparing with other studies, the species of the mite was reported as *Cnemidocoptes pilae*. The necropsy was done according to the classic method and necessary samples were taken. There were no lesions in internal organs but minor hyperemia was observed. The sciatic and brachial nerves were examined and no deformity was observed in these nerves. Brain was pulled out by cutting the skull and samples were taken from cerebra and cerebellum. Hyperemia was also observed in the brain. There were no other abnormalities in brain's cross-section.

Keywords: Budgerigars, pathology, cnemidocoptes

S3- 0019 The adiponectin receptors are expressed in chicken osteoblast

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Adiponectin (ADPN) has been considered to be synthesized and secreted by the adipose tissue, and is reported to have an influence on glucose utilization, insulin sensitivity, and energy homeostasis. It has also been demonstrated that ADPN and its receptors, AdipoR1 and AdipoR2, are expressed in mammalian osteoblasts and correlate with bone metabolism. Some researchers have showed that body fat can have effects on chicken bone mineral density; however, the mechanisms of which are unknown. Therefore, it would be interesting to study the role of ADPN to help with prevention and control of chicken bone disorders such as laying hens osteoporosis. To detect whether or not the ADPN and its receptor are expressed in chicken osteoblast cells, chicken osteoblasts were cultured using 15-day chick embryos. The phenotype of cells was identified by alkaline phosphatase staining and the expression of osteocalcin mRNA. The ADPN, AdipoR1 and AdipoR2 of chicken osteoblasts were detected by RT-PCR and western blotting, and the relative value of AdipoR1 and AdipoR2 mRNA were analyzed by real-time PCR. The results showed that the cultured cells were positively stained by alkaline phosphatase and that osteocalcin is highly expressed in these cells. Our research showed that ADPN receptors including AdipoR1 and AdipoR2 are expressed in chicken osteoblast cells, and AdipoR2 has higher expression value than AdipoR1. However, although present in mammalian osteoblasts, expression of ADPN was not found in chicken osteoblasts. The function of adiponectin on bone metabolism still remains unclear, and our findings suggest that adiponectin might have effects on chicken osteoblast proliferation and differentiation, and that the regulatory mechanism of adiponectin on osteoblasts might be different when compared with mammals. **Keywords:** adiponectin, AdipoR1, AdipoR2, osteoblast, chicken

S3-0020 Effect of vanadium and tea polyphenols on intestinal morphology, microflora and short-chain fatty acid profile of laying hens

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Vanadium (V) is a trace element which can induce dysfunction of gastro-intestine and egg quality deterioration of laying hens. This study was conducted to determine the effect of tea polyphenols (TP) on intestinal morphology, microflora and short-chain fatty acid (SCFA) profile of laying hens fed vanadium-containing diets. A total of 120 Lohman laying hens (67-wk-old) were randomly divided into 4 groups with 6 replicates of 5 birds each for a 35-day feeding trial. The dietary treatments were as follows: (1) control (CON), fed a basal diet; (2) vanadium treatment (V10), CON + 10 mg V/kg; (3) TP treatment 1 (TP1): V10 + 600 mg TP/kg; (4) TP treatment 2 (TP2): V10 + 1000 mg TP/kg. Fed 10 mg V/kg diets to laying hens didn't affect the caecum flora diversity index (H), degree of homogeneity (EH) and richness (S), but hens fed TP2 diet decreased the H, EH and S ($P < 0.05$). The cecum acetic acid concentration was lower in V10 treatment and higher in TP2 treatment ($P < 0.05$). Addition of 10 mg/kg V resulted in an increased ($P < 0.01$) duodenal cell apoptosis rate, 1000 mg/kg TP supplementation overcame ($P < 0.01$) this reduction effect induced by vanadium. The results indicate that supplementation of 10 mg/kg vanadium increased duodenal cell apoptosis and reduced cecum SCFA content. Addition of 1000 mg/kg TP decreased the SCFA production to affect cecum flora ecology, and protected the duodenal cell apoptosis and cecum decrease caused by vanadium.

Keywords: vanadium, tea polyphenols, apoptosis, cecum microflora, duodenum morphology, laying hens

S3- 0021 Molecular characterization and phylogenetic study base Nucleocapsid gene of avian infectious bronchitis virus isolated from broilers farm, 2014-2015

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Avian infectious bronchitis (IB) is an economically important poultry disease. The emergence of new infectious bronchitis virus genotypes has complicated IB control programs. This is the first molecular analysis of the “N” gene of Iranian IBVs. The nucleocapsid gene of ten IBV isolates (which belonged to four different genotypes) was amplified using specific primers. The phylogenetic trees were constructed based on nucleotide and amino acid sequences of “N” gene. Results: IBV genotyping based on “N” gene showed similar IBV classification which was obtained from spike gene analysis and ten isolates were belonged to Massachusetts, QX, 793/B and variant-2 genotypes. Different strains had 89.97-99.75% homologies in their amino acid sequences. The highest nucleotide sequence similarity was observed between IBKG-1 and IBKG-8 (793/B type IBVs), while the lowest was seen between IBKG-5 and IBKG-9 (QX- type and Variant-2 type) IBV isolates. This low similarity is of an interest because the N protein is highly conserved among different IBV strains. “N” Protein structural analysis revealed that the isolates has 8 to 10 alpha helices and 6 to 8 beta sheets. RNA binding regions with the specificity of more than 80% were investigated. The present study provided basic information to develop recombinant nucleocapsid proteins that are applicable in rapid diagnostic tests and ELISA, recombinant vaccines. In addition, it can be concluded that the nucleocapsid gene of IBV is useful in molecular epidemiology studies.

Keywords: avian infectious bronchitis, nucleocapsid, phylogenetic, characterization, Iran

S3- 0022 Genotyping of infectious bronchitis virus isolates in Iran, 2015: An Update Data

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Infectious bronchitis (IB) is a viral avian disease with economic importance in the world, including Iran. S1 gene sequencing has been used for molecular epidemiological studies and genotypic characterization of infectious bronchitis virus (IBV). A total of 100 IBVs were isolated from the tissue samples of clinically suspected chickens of Iranian broiler farms. The isolates were confirmed by real-time polymerase chain reaction (PCR) and characterized by sequencing the spike glycoprotein gene. Results: The isolates formed four distinct phylogenetic groups [IS/1494/06 (Var2) like, 4/91 like, QX like, and Mass like]. The most frequently detected genotype were Var2-like (IS/1494/06 like) viruses with a total prevalence of 54%. This study demonstrates a constant evolution of IBV in Iran, demonstrating the need of continuous monitoring and development of new vaccines based on indigenous viruses.

Keywords: genotyping, Iran, phylogenetic study, spike, infectious bronchitis virus

S3- 0023 Effect of route of inoculation on replication of avian influenza virus (H9N2) and interferon gene expression in Guinea fowls (*Numida meleagris*)

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The study was conducted to investigate replication of a reassortant H9N2 avian influenza virus (AIV) and induction of interferon (IFN γ) response after aerosol or intranasal inoculation with the virus in guinea fowls. To determine virus shedding pattern, oropharyngeal and cloacal swabs, and tissue specimens of trachea, lungs, spleen, and caecal tonsils were collected postinfection. Infected guinea fowls showed mild clinical signs, while control negative guinea fowls remained healthy and active throughout the experiment irrespective of the inoculation route. However, the clinical signs were more prominent in guinea fowls infected through aerosol route. Virus was detected in all oropharyngeal and cloacal swabs up to 8 dpi in guinea fowls from both inoculation groups. However, virus was detected more frequently ($P < 0.05$) and in higher titres in oropharyngeal swabs and specimens of trachea and lungs from the group exposed to aerosols than from the group given intranasal drops. In accord with viral replication findings, expressions of IFN γ were up-regulated to a significantly higher level ($P < 0.05$) in lung tissue specimens from the group exposed to virus aerosol than from controls that were given PBS intranasally on 1, 2 and 4 dpi. On the other hand, IFN γ was up-regulated ($P < 0.05$) above that of controls in lung tissue specimens from the group given intranasal drops of virus only on 4 dpi. These findings indicate that virus administered in aerosols was more efficient than virus administered as intranasal drops, in infecting the lower respiratory tract and in inducing the activity of the IFN γ gene. The results of this study suggest that virus aerosols might cause intense respiratory infection and increase the shedding of the H9N2 AIV in guinea fowls, highlighting the potential role of guinea fowls as mixing bowl for transmission and maintenance of H9N2 AIV between premises.

Keywords: avian influenza virus, aerosol, viral replication, interferon, Guinea fowls

S3- 0024 Generation and protective efficacy of a cold-adapted attenuated avian H9N2 influenza vaccine

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To prevent H9N2 avian influenza virus infection in chickens, a long-term vaccination program employing inactivated vaccines has been implemented in China. However, the protective efficacy of inactivated vaccines against antigenic drift variants is limited, and H9N2 influenza virus continues to circulate in vaccinated chicken flocks in China. Therefore, developing a cross-reactive vaccine to control the impact of H9N2 influenza in the poultry industry remains a high priority. In the present study, we developed a live cold-adapted H9N2 influenza vaccine candidate (SD/01/10-ca) by serial passages in embryonated eggs at successively lower temperatures. A total of 13 amino acid substitutions occurred during the cold-adaptation of this H9N2 virus. The candidate was safe in chickens and induced robust hemagglutination-inhibition (HI) antibody responses and influenza virus-specific CD4⁺ and CD8⁺ T cell immune responses in chickens immunized intranasally. Importantly, the candidate could confer protection of chickens from homologous and heterogenous H9N2 viruses. These results demonstrated that the cold-adapted attenuated H9N2 virus would be selected as a vaccine to control the infection of prevalent H9N2 influenza viruses in chickens.

Keywords: influenza virus, H9N2, vaccine, chicken

S3-0025 Application of immunostimulator "Poliferon" for broiler chicks

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The efficiency of immunostimulator "Poliferon" was studied in All-Russian Research and Technological Poultry Institute on cage-housed broiler chicks Cobb-500 since 1 to 37 days of age. The preparation contains affinity purified antibodies to human gamma-interferon, CD4 and C-terminal fragment of beta-subunit of insulin receptor in release-active form. Day-old broilers were allotted in 4 groups, 70 birds per group. In first 3 groups (experimental) broilers were treated with fresh solutions of "Poliferon" with drinking water according to the following schemes: in Group 1 - daily, from 1 to 37 days of age (37 days totally); in Group 2 - periodically, from 1 to 5, from 15 to 20 and from 30 to 35 days of age (15 days totally); in Group 3 - daily, from 27 to 37 days of age (10 days totally). The solutions were prepared on the basis of 0.005 g of the preparation per bird daily. In Group 4 (control) broilers were fed fresh solution of placebo according to the same scheme as Group 1. The results showed that average live BW at 37 days of age in Group 2 was significantly higher (by 96.39 g or 6%, $P < 0.05$) compared to control; in Group 3 higher by 68.87 g or 4.2%, $P < 0.05$. In Group 1 this improvement was insignificant but also substantial (by 3.1%). Mortality levels in experimental groups were 1.4% lower compared to control. The conclusion was made that preparation "Poliferon" with drinking water significantly improved live BW and mortality in broiler chicks. The preparation is well tolerated and entirely safe for poultry.

Keywords: "Poliferon" preparation, broiler chicks, mortality, live bodyweight

S3-0027 The mutation of PA-K356R lowers the barrier to cross-species transmission of H9N2 avian influenza viruses into mammalian hosts

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Previous computational studies suggest a host-specific PA-356 residue possessing conserved lysine (K) in avian viruses and arginine (R) in human strains, and the mutation of K356R present in H7N9's PA genes now. However, the biological role of the mutation PA-K356R remains poorly understood. In this study, through investigating all available PA gene sequences, we found an adaptive mutation trace from avian-like signature K to human-like R in position PA-356 since avian-origin H1N1 virus caused the first pandemic in human in 1918. Strikingly, the mutation PA-K356R has emerged in recent avian H9N2 influenza virus and quickly become predominant. By reassortment, PA-K356R is delivered to the genomes of H9N2's reassortants. After jumping to humans, PA-K356R alone, or coupled with the well known mammalian adaptive mutation PB2-E627K, exhibit higher prevalence in H7N9 and H10N8 human viruses instead of avian strains. Further characterization of PA-K356R genotype revealed that this mutation contributed to nuclear import of PB1-PA dimer of avian H9N2 virus, enhanced polymerase activity, and augmented viral genome transcription and replication in human A549 cells. Consequently, H9N2 virus carrying this residue replicated to a significantly higher titer in human cells, and caused enhanced infection and pathogenicity in mice with elevated inflammatory response. The effect of PA-K356R is comparable to that of PB2-E627K, suggesting that the PA mutation might overcome the restriction associated with an avian PB2 in human. Co-mutation of PA-K356R and PB2-E627K exhibited synergic effect in infection of mammals, which might enhance the pathogenicity of H7N9/H10N8 viruses in humans. Collectively, our study found that PA-356R is a novel marker of mammalian adaption, which lowers the barrier of H9N2 avian virus or might lowers the barrier of its reassortants to cross-species transmission and might facilitate the pathogenicity in humans by in coordination with PB2-E627K.

Keywords: H9N2, avian influenza virus, PA-K356R, host adaptation, pathogenicity

S3-0028 Amino acid substitutions in PB2 and NA contribute to the pathogenicity and neurovirulence of a mouse-adapted H10N7 influenza A virus in mice

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H10 subtype influenza viruses have been circulating worldwide in multiple avian species and have repeatedly infected mammals to cause typical disease. The occasionally avian-to-human interspecies transmission of H10 viruses raises concerns about the possibility of viral adaption with increased virulence for humans. Particularly, the fatal human infections with a novel avian-origin H10N8 influenza virus in China were reported recently. To investigate the genetic basis of H10 influenza virus host range and pathogenicity in mammals, we generated a mouse-adapted H10N7 virus (BJ27-MA) that possessed significantly higher virulence than wide-type virus (BJ27). Amino acid substitutions in PB2 (E158G and M631L), HA (G218E), and NA (K110E and S453I) occurred in BJ27-MA. Assessments of pathogenicity and replication abilities in vivo and in vitro showed that substitutions of E158G and M631L in PB2, K110E and S453I in NA significantly increased viral pathogenicity and replication in mice and mammalian cells; and single amino acid substitution of M631L in PB2 plays key roles in the adaptation of H10N7 virus in mammal. Particularly, the combination of PB2 and NA from BJ27-MA contributed viral neurovirulence in mice. Meanwhile, mutations in PB2 and NA were found to significantly upregulated viral polymerase activity and NA enzymatic activity respectively. Thus, our results suggest that mutations of E158G and M631L in PB2, K110E and S453I in NA are critical for the increased pathogenicity of H10N7 influenza virus in mammalian host, which might also pose a threat to human health.

Keywords: H10N7 influenza virus, mammal adaptation, pathogenicity, neurovirulence

S3-0029 Antigenic characterization and cross-protection of co-circulating antigenic variants for H5N1 highly pathogenic avian influenza viruses in Indonesia

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The aims of our research were to characterize the antigenic profile of H5N1 highly pathogenic avian influenza viruses (HPAIV) in Indonesia and to further evaluate the extent of cross protections between the antigenic variants to be detected in Indonesia. A total of 15 H5N1 HPAIVs from domestic poultry from 2007 to 2015 were used in antigenic characterization, and antigenic cartography was constructed for these viruses based on chicken reference sera derived hemagglutination inhibition (HI) data. Results showed that the viruses were antigenically distinct, and at least two H5 antigenic groups (clade 2.1.3 and 2.3.2) were co-circulating in Indonesia. Two representative isolates, A/Env/Ciamis/2013 (H5N1) (clade 2.1.3) and A/Ck/Serang2/2015 (H5N1) (clade 2.3.2), were selected for cross protection experiment. These two testing strains has an antigenic distance 2.46 units, each unit equal to a 2-fold change in HI data. A total of 40 specified pathogen free (SPF) chicken were separated into four groups, 10 bird per group. The birds in groups 1 and 3 were vaccinated once with A/Env/Ciamis/2013 (H5N1) at four weeks of age, and the birds in group 2 and 4 were remained unvaccinated as mock. Four weeks after vaccination, birds in groups 1 and 2 were challenged using A/Env/Ciamis/2013 (H5N1) whereas those in groups 3 and 4 using A/Ck/Serang2/2015 (H5N1). Clinical sign and mortality were monitored for eight days postchallenge. Pre-challenge antibodies, mortality and morbidity were used as parameters of protection. Our vaccine-challenge study showed that 100% vaccinated chickens challenged with homologous virus survived whereas only 30% those with heterologous virus did. In summary, our study suggested there are antigenic variations among the H5N1 viruses in Indonesia and the two co-circulating antigenic variants (clade 2.1.3 and 2.3.2) provided a poor cross protection. This study can provide a guidance in vaccine strain selection in Indonesia.

Keywords: H5N1 HPAIV, antigenic variants, antigenic cartography, antigenic distance, cross protection

S3- 0030 Postvaccinal immunity in broiler chicks treated with Poliferon preparation

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The influence of Poliferon preparation on post-vaccinal immunity in Cobb- 500 broilers was studied; this preparation contains affinity purified antibodies to human gamma- interferon, CD4 and C-terminal fragment of beta-subunit of insulin receptor in release-active form. The preparation was fed with drinking water (on the basis of 0.005 g/bird/day). Broilers were allotted in 3 groups, 70 birds in each. Group 1 was treated with Poliferon daily from 1 to 37 days of age; Group 2 was treated periodically from 1 to 5, from 15 to 20 and from 30 to 35 days of age (15 days totally); Group 3 (control) was treated with placebo daily from 1 to 37 days of age. The results showed that average titer of antibodies against Newcastle disease was in Group 1 treated with Poliferon daily: higher than in control by 6.8% and higher by 23.7% than in periodically treated Group 2. The percentage of positive tests was also maximal in Group 1. Average antibody titers against Gumboro disease were high in all 3 Groups; this parameter in Group 1 exceeded Group 2 by 28.5%. The trial proved immunomodulating effect of Poliferon in broiler chicks treated with live vaccines. The formation patterns of immunity against main avian pathogens were found to be optimal after both daily and periodical application of Poliferon.

Keywords: broiler chicks, poliferon, immunity

S3-0031 Characterization by virtual restriction fragment length polymorphism (RFLP) analysis of recent Algerian isolates of infectious bursal disease virus (IBDV)

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Clinical outbreaks of Infectious Bursal Disease (IBD) continue to be reported in correctly vaccinated flocks. These vaccination failures are probably related to a significant increase in virus virulence. The reverse transcriptase- polymerase chain reaction/restriction fragment length polymorphism (RT-PCR/RFLP) was used to characterize 5 wild infectious bursal disease viruses (IBDV) originating from outbreaks reported in Northeast of Algeria. A 743 bp fragment in the VP2 gene was amplified, sequenced and then submitted to virtual enzyme digestion using Geneious software. The RFLP profiles of the 5 studied strains, determined using BstNI and MboI restriction enzymes, were compared with previously published profiles. The restriction by the BstNI enzyme has generated the same profile for the 5 studied strains while 2 profiles were generated by the MboI restriction endonuclease, confirming that BstNI enzyme is less efficient in discriminating between viruses. The 2 different RFLP generated by the pair of enzymes were unique and none of them was consistent with the previously published RFLP. The results of this study confirm that IBDV are continuously evolving.

Keywords: RT-PCR/RFLP, IBDV, virtual digestion, Algeria

S3-0032 Phylogenetic and histopathological characterization of Newcastle disease viruses reveals high prevalence of very virulent strains of genotype VIIId in Egypt

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Newcastle disease virus (NDV) is a highly contagious disease and a major challenge for commercial poultry industry in Egypt. Devastating outbreaks endangered chicken broiler farms and backyards in different provinces during 2013 and 2014. Emerging need was generated for phylogenetic and pathological characterization of NDV strains mostly circulated during outbreaks. Experimental design: In this study clinicopathological examination of morbid birds were carried out. Mouse monoclonal anti NDV nucleoprotein has been used for immunohistochemical diagnosis. Genomic viral RNA has been extracted for RRT - PCR, updated designed primers for full fusion gene amplification was produced. Purified RT-PCR products were sequenced then phylogeny for the sequenced samples with reference and Egyptian strains previously published and available on the GenBank database using MEGA 6. An alignment analysis of deduced amino acids and nucleotides sequences of the sequenced fusion gene were created using the CLUSTAL W Multiple Sequence Alignment Program. Clinicopathological examination revealed velogenic viscerotropic ND features and positive staining for ND virus (NDV) antigen in pantropic organs. The resulted sequence showed motif ¹¹²RRQKRF¹¹⁷ which is indicative of velogenic character NDV strains, and indicate presence of 2 mutations E74D and D170N in two strains. Phylogenetic analysis revealed that Egyptian strains are closely related to NDV genotype VIId strains of south East Asian countries based on the high nucleotide and amino acid similarity. In conclusion, data obtained in the present study confirm high circulation of NDV genotype VIId which became the predominant strain and causing devastated outbreaks in poultry farms. Those NDV VIId strains might be originated from Southeast Asian countries. Continuous monitoring of the flocks' immunological status should be carried out to evaluate the antibody response to currently used vaccines.

Keywords: NDV VIId, RRT-PCR, full fusion gene sequence, immunohistochemistry, molecular evolution

S3- 0033 Comparative study of AviPro[®] IBD Xtreme, AviPro[®] Precise and Cevac Transmune[®] and assessment of potential complementary dual programs in broilers

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The aim of this study was to compare ELISA titer development and bursa lesions of single vaccination with AviPro[®] IBD Xtreme, AviPro[®] Precise or IBD immune complex Cevac Transmune[®] (groups 1, 2 and 3) versus immune complex plus AviPro[®] IBD Xtreme (group 4) or immune complex plus AviPro[®] Precise (group 5). At day of hatch, commercial broilers were individually identified and housed in 6 isolators based on the different treatments including a negative control group. At day 0, groups 3, 4 and 5 were vaccinated s.c. with immune complex. At 14 days of age, groups 1 and 4 were vaccinated with AviPro[®] IBD Xtreme. At 18 days of age, groups 2 and 5 were vaccinated with AviPro[®] Precise. At day 14, 18, 21, 25, 28, 32, 35 and 39 samples for serology were taken. At day 39 all birds were euthanized and from five birds per group bursas were collected for histological examination to determine the Bursal Lesion Score (BLS). The mean BLS at day 39 was 2.0 (AviPro[®] Precise alone), 3.0 (AviPro[®] IBD Xtreme alone), 3.2 (immune complex alone), 3.2 (group 5) and 3.4 (group 4). Regarding the comparison of antibody response of group 3 and 4, significant difference could be noticed in percentage of birds with already higher titers of >2000 at day 21 and 25 of age. At day 21, percentage of birds with titers higher (P=0.05, X² test) than 2000 is 10% (both ELISA's) in group 3 compared to 50% (both ELISA's) in group 4. At 25 days, this difference in percentage of positive birds is still 20% (IDEXX) and 10% (BioChek) higher in group 4. This indicates that at least a part of the birds started to respond earlier in group 4 than group 3, which is a strong indication of earlier protection. The results of this study demonstrate that combined use of AviPro[®] IBD Xtreme and immune complex induce earlier protection than immune complex alone. This combination might be beneficial in certain field situations to ensure fast immunization and early protection against Gumboro.

Keywords: Gumboro, vaccination, protection, broilers

S3-0034 Integration of reticuloendotheliosis virus in pox virus causing mortality in pox disease of avian species

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Reticuloendotheliosis virus (REV) is a provirus with oncogenic property belongs to family Retroviridae while Avipoxvirus (APV) is a member of the Poxviridae family within the sub family Chordopoxvirinae. It causes pox disease affecting over 232 species in 23 orders in birds like Poultry, Turkey, Pigeon etc. The disease is manifested in two forms – cutaneous and diphtheritic form. Here we present cases of pox disease in Poultry, Pigeon, Duck and Turkey with varying degree of clinical lesions (both cutaneous and diphtheritic form) and high mortality from Northeast India. The poxvirus was confirmed by amplifying P4B gene of APV through polymerase chain reaction (PCR) and the REV integration into the pox genome by the presence of LTR gene at insertion site. The env gene of REV was also detected leading to further investigation for the whole genome inserts. The PCR- Restriction Fragment Length Polymorphism (RFLP) of P4b gene clearly differentiates Pigeon poxvirus from poxvirus of Poultry, Duck and Turkey. The integration of REV is partial with varying length of gene sequence in poxvirus from Duck and Turkey while whole genome insertion was detected in poxvirus from Poultry. No REV integration could be detected from poxvirus of Pigeon. Recurring pox infection with greater intensity of clinical lesion and mortality in the subsequent year was observed in Turkey and Poultry. The REV genome insertion of various fragments was found to be correlating with the degree of severity of disease leading to speculation the integration is a gradual process and reflecting virulence alteration. The expression of one or more of the acquired retroviral genes could alter the biological properties of the host and result in the emergence of a modified virus where the current vaccines would not afford adequate protection. The situation warrants further studies since the outbreak of pox disease in avian species is frequently reported in spite of effective vaccine available for its control.

Keywords: avian pox virus, reticuloendotheliosis virus, pox disease

S3-0035 Evolution of infectious bronchitis virus in China over the past two decades

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Avian infectious bronchitis is a highly contagious disease caused by infectious bronchitis virus (IBV) that affects poultry production worldwide. Vaccines have been shown to impose strong selection pressure on the evolution of circulating viruses. They may also give rise to new strains through recombination, allowing the viruses to escape host defenses and evolve further. To understand the population dynamics of the dominant IBV genotypes in China, here we designed a study to measure the evolution dynamics of IBV strains in China. We investigated the evolutionary trends of the main endemic QX genotype and the Mass vaccine-like genotype in China during 1990s-2010s by analyzing a dataset of 1022 complete sequences of the spike gene (S1) from viruses isolated at different times. We then estimated the evolution rate from a data set of 107 full-length IBV genome sequences by Bayesian methods. Using the Strict Clock method, we estimated the earliest time of IBV circulation. We also identified the genes that played a dominant role in IBV evolution by testing the positive selection pressure of different genes. The phylogenetic dissimilarity of different gene trees in the data set indicated possible recombination. Fourteen isolates were identified as recombinants, possibly generated from vaccines of the Massachusetts serotype in recombination with circulating viruses. The earliest IBV in China was found to have existed in the early 1900s, and continues to evolve at a rate of approximately 10-5 substitutions/site/year. We found that, purifying selection is the main evolutionary pressure in the protein-coding regions, while the S1 gene bears the greatest positive selection pressure. The proportion of QX-like genotype strains increased over time. These results indicate that the genotypes of Chinese IBVs have undergone a remarkable transition during the past 20 years.

Keywords: infectious bronchitis virus, evolution, selection pressure

S3-0036 Characterization and analysis of an infectious bronchitis virus (IBV) strain isolated from southern China in 2013

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Infectious bronchitis (IB) is a severe diseases in fowl flocks all around the world and caused by infectious bronchitis virus (IBV). To understand the mechanisms behind the evolution of IBV and the emergence of new variants will be an imperative point in the future control of the disease. GD strain was isolated from southern China in 2013 and the complete genome sequence was detected. Sequences of full-length genomes and structural genes were compared with other reference IBVs. Deduced amino acid sequences were aligned and the phylogenetic trees were mapped with the full-length genomes and structural genes. Then the recombination detection of complete genome sequences of the IBV GD strain and other reference strains were taken. All the sequences of S1 gene of IBVs until the November 2015 based on the data released from NCBI were collected and the phylogenetic analysis was taken to cluster the TW- type group. The proportions of TW-type strains in all the isolations every years were computed. The genome is approximately 27,680 nt in length, comprising six genes. There were insertions, mutations in most of the structure gene. The S1 gene has a highest identify to TW2575/98 strain (98.3% nt and 96.8% aa sequence identity) isolated in Taiwan and has a far distant from H120 vaccine. Phylogenetic analysis showed that S1 gene of GD was also relative to TW-type strains. Recombination analysis indicated that GD was a chimera whose putative parental strains belonged to the QX- and TW-type subgroups. We also concerned that an increasing number of TW-type strains had been isolated successively in China mainland in recent years. The results of sequence and phylogenetic analysis of GD strain has shown that the new isolation is probably a recombinant strain between the QX- type and TW- type strains and the emergence of new TW- type strains has been increasing in southern of China during the recent years.

Keywords: infectious bronchitis virus, China, sequence analysis, phylogenetic analysis, recombination

S3-0037 Contribution of HN protein length diversity to Newcastle disease virus virulence, replication, and biologicalactivities

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The hemagglutinin-neuraminidase (HN) protein of Newcastle disease virus (NDV) plays important roles in viral invasion and maturation, with three distinct activities: receptor binding, NA activity (receptor cleavage), and fusion promotion. The HN protein has been shown to contribute greatly to NDV pathogenesis. It was found that the HN proteins vary in length, and at least nine different length variants have been reported to date. To evaluate the contribution of length diversity in the hemagglutinin- neuraminidase (HN) protein to the pathogenicity and biological characteristics of Newcastle disease virus (NDV), we used reverse genetics to generate a series of recombinant NDVs containing truncated or extended HN proteins based on an infectious clone of genotype VII NDV (SG10 strain). The mean death times (MDT) and intracerebral pathogenicity indices (ICPI) of these showed that the different length mutations in the HN protein did not alter the virulence of NDV. In vitro studies of recombinant NDVs containing truncated or extended HN proteins revealed that the extension of HN protein increased its receptor-binding ability and impaired its neuraminidase activity, fusogenic activity, and replication. Our results demonstrate that the HN biological activities affected by its C-terminal extension are associated with NDV replication but not its virulence. The balance between the three functions of HN determines the biological characteristics of NDV. These findings may also be applicable to other paramyxoviruses.

Keywords: Newcastle disease virus, HN, length diversity, virulence, replication

S3-0038 Polymerase-associated proteins are associated with the virulence of Newcastle Disease Virus

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Newcastle disease virus (NDV) can cause severe disease in chickens. Most recent chicken-derived NDV isolates of China belong to genotype VIIId, of which are velogenic strains. The genotype II strain LaSota is widely used as commercial live vaccine. With a long period of intense vaccination, there exists the possibility of genetic recombination between the wild type and vaccine strains, which may affect the virulence of NDV. In this study, we evaluated the role of polymerase-associated proteins (NP, P, L) played on the virulence of NDV, thus to investigate the molecular mechanisms of this phenomenon. Using reverse genetics, the NP, P and L genes were exchanged individually and in different combinations between a highly virulent NDV strain SG10 (VIIId) and LaSota. A total of 13 chimeric viruses were constructed and evaluated for their pathogenicities in chicken embryos and 1-day-old chickens. Viral genome replication kinetics was examined *in vitro*. Our results showed that the pathogenicities of the NP, P, or NP-P transfer chimeric viruses were similar to their respective parental viruses. However, replacement of the L gene alone altered the pathogenicity of the chimeric virus slightly, and replacement of the NP-L, P-L, NP-P-L simultaneously combinations altered the pathogenicities of the related chimeric viruses significantly, especially in replacement of the polymerase-associated proteins (NP-P-L). The change in the replication kinetics of each chimeric virus was similar to the change of its virulence, indicating that the change in pathogenicity may be correlation with the changed replication level of the chimeric virus. In summary, among the polymerase-associated proteins, the N and P genes probably play minor roles in virulence, and the L gene contributes most to the viral virulence and can be enhanced by the presence of homotypic NP and P proteins. These findings make us have a new understanding on the pathogenesis of NDV infection.

Keywords: NDV, genetic recombination, virulence

S3-0039 Expression of IBV genes in mycoplasma synoviae strain of MS-H

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Mycoplasma synoviae (*M. synoviae*) is an important avian pathogen infecting chickens, turkeys and some other avian species, causing subclinical respiratory tract infection and synovitis, and some strains cause "glass top eggs". The severity of clinical respiratory symptoms associated with *M. synoviae* can be aggravated by co-infection with different respiratory pathogens such as infectious bronchitis virus (IBV). The MS-H live attenuated temperature-sensitive (ts-) vaccine (Vaxsafe MS[®], Bioproperties Ltd., Ringwood, Victoria, Australia) has been approved in Australia since 1996 and is now widely used in a number of countries to control and prevent infection with *M. synoviae*. The aim of this study was to use the MS-H vaccine as a vector to develop a recombinant vaccine for MS and other common pathogens such as infectious bronchitis virus which colonises upper respiratory tract. OriC vectors containing partial the infectious bronchitis virus (IBV) spike glycoprotein S1 and nucleocapsid protein N genes were constructed and used to transform *M. synoviae* vaccine strain of MS-H. The vector, carrying the complete *dnA* gene along with upstream and downstream *DnaA* boxes, was successfully introduced into *M. synoviae* and was able to freely replicate, but also integrated into the chromosomal *oriC* region. The *vlhA* promoter was used to drive the expression of S1 and N genes. IBV RNA and protein expression in *M. synoviae* will be examined by real-time PCR and western immunoblotting respectively. The RNA expression of partial IBV S1 gene was detected in the MS-H transformant (pMS-IBV S1). Expression of the IBV S1 and N will be examined by western immunoblotting. A potential vaccine candidate for prevention of MS and IBV was developed. Also, it is anticipated that this system is likely to be suitable for the expression of viral genes in *M. synoviae*.

Keywords: mycoplasma synoviae, IBV, recombinant vaccine

S3-0040 A cross-protective avian influenza vaccine candidate based on extracellular domain of M2 protein expressed by transgenic *Eimeria tenella*

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Avian influenza virus not only cause huge economic loss in poultry industry but there is also high risk of transmission to human via mucosal system. A cross-protective avian influenza vaccine is considered to be a successful intervention strategy in controlling avian influenza and a preferable way to prevent human from influenza virus infection. Transgenic *Eimeria tenella* lines are powerful tools deliver exogenous proteins to the intestinal mucosal immune system and the exogenous antigens targeted to different cellular compartments could elicit dichotomic immune responses in chickens. Here, we constructed a transgenic *E. tenella* line, EtpM2e, by transfection with plasmid encoding 6 tandem-repeated copies of extracellular domain of M2 protein. By pyrimethamine selection and fluorescence activated cell sorting, the obtained EtpM2e line was identified by indirect immunofluorescence assay and western blotting. After triple oral immunization with EtpM2e oocysts in SPF chickens, IgY antibody in serum, as well as virus specific IFN- γ secretion T lymphocyte in PBMCs against H9N2 and H5N1 and were detected by ELISA and ELISPOT, respectively. Results showed that a dominant cross-reactive antibody response in serum against H9N2 and H5N1 virus antigen in EtpM2e immunized birds than the controls. Moreover, virus specific cellular immune response in immunized birds, the amount of H9N2 and H5N1 virus-specific IFN- γ secretion T lymphocyte in PBMCs, was significantly higher than those non-immunized birds. These results indicated that EtpM2e induced cross-protective immunity against different influenza virus types and could be further developed as a novel avian influenza vaccine. (This work was supported by the National Natural Science Foundation of China (31330076, 31472180)).

Keywords: transgenic *Eimeria tenella*, avian influenza virus, M2e, cross-protective immunity

S3-0041 Host cell interactome of PA of H5N1 influenza A virus in chicken cells

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Influenza A virus relies heavily on host cellular proteins during the course of infection, and therefore, host proteins that have closely physical interactions with viral proteins have crucial impact on virus virulence. Host factor are comprehensively involved in viral life cycle, including uncoating, transcription, viral RNA transportation and packaging. Host factor may exert either promotive or suppressive influence on virus infection, depends on the specific role of host protein. Here we report the interaction landscape of PA protein of H5N1 IAV in chicken cells by affinity purification and mass spectrometry. Exogenous FLAG-tagged PA was expressed in chicken embryo fibroblast cells, PA interacting complex was captured by co-immunoprecipitation and analyzed by mass spectrometry. Totally 138 proteins were identified as PA-host interacting factors, many of which were involved in crucial biological processes. Most of subunits of several protein complexes including the minichromosome maintenance complex (MCM), 26S proteasome and the coat protein I (COPI) complex, associated with PA in chicken cells, indicating the essential role of these functional protein complexes during the course of IAV infection. Typically, both Gene Ontology and pathway enrichment analysis showed strong enrichment of PA interacting proteins in the category of DNA replication, covering genes such as PCNA, MCM2, 3, 4, 5, and 7. Many studies has been carried out on elucidation of the viral-host interactions and their functional mechanisms in human, the global viral-host interactions of avian flu in chicken cells and still need to be further explored. This study has uncovered the comprehensive interactome of PA of H5N1 IAV in its chicken host and lay the foundation for further investigation into the newly identified viral-host interactions.

Keywords: chicken, PA, H5N1, AP-MS

S3- 0042 Isolation and molecular characterization of novel Newcastle disease virus isolates in India

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Newcastle disease virus (NDV) is a highly contagious avian disease with worldwide distribution. Despite the extensive use of vaccines, several outbreaks of NDV have been reported in India. In the present study we isolated a novel NDV strain from different outbreaks with up to 40% mortality in commercial broiler chickens from the eastern part of India in 2014-15. On post mortem examination we found hydropericardium, pale and mottled livers, swollen kidneys, ascitis and few birds with visceral gout. NDV was isolated from pooled tissue samples (spleen, liver and kidneys) in chicken embryo fibroblast and chicken embryo liver cells. Rounding, aggregation, syncytia, and detachment of cells was observed on second passage. Virus was not recovered by inoculating embryonated specific pathogen free (SPF) eggs. As the viral titre obtained in cell culture was low, additional passages were carried out in embryonated eggs. The mean time to death was 48 hours. The intracerebral pathogenicity index values range from 1.76 to 1.90 confirming the velogenic nature of the NDV isolates. Sequencing of the fusion cleavage site identified multiple basic amino acids (112 RRQKRF 117 motif), confirming this to be a virulent isolate. A phylogenetic tree was constructed based on the complete F gene and HN gene showing a close similarity with genotype XIIIb. One-day-old and 4-week-old SPF chickens were inoculated intraocularly with PDRC\15\EZ\3\NDV isolate and 90% mortality was observed between 3 to 5 day post inoculation. Nephrosis, necrohemorrhagic lesions and swelling of the kidneys the main lesion. Quantitative reverse transcriptase polymerase chain reaction analysis of kidney, spleen, liver and trachea samples at 3-5 days post inoculation showed the highest number of virus particles in kidneys followed by spleen, liver and trachea. In conclusion this study showed that velogenic NDV strains belonging to genotype XIIIb are circulating in the east zone of India.

Keywords: NDV, genotype, pathogenicity, virulent, MDT

S3- 0043 Chicken embryo lethality assay for determining the virulence of avian *Enterococcus faecalis* isolates

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Enterococcus faecalis (*E. faecalis*) is the most isolated bacterium in field cases of amyloid arthropathy (AA amyloidosis) in chickens, resulting in a great negative impact on pullet growth. Hence, the Embryo Lethality Assay (ELA) is proposed in the present study as a tool to evaluate the virulence of *E. faecalis* field strains. The ELA was performed ten times with subsets of 6-7 *E. faecalis* strains each on a sample of 9,987 eggs of white layers including controls. In each trial, a total of 100 embryonated eggs were inoculated with one strain. 3 to 24 colony-forming units were inoculated into the allantoic cavity of 10 day-old embryos. The mortality rate of the embryos was determined by means of candling the eggs over a period of 7 days. The ELA was able to distinguish the virulence of the *E. faecalis* strains. 28 *E. faecalis* strains were considered as avirulent strains with a mortality rate of below 40 %. Only a single strain was highly virulent with a mortality rate of 81 %. The remaining 39 strains were classified as strains of moderate pathogenicity whose mortality rate varied from 40 to 75 %. The most severe changes were sepsis, hemorrhages and subcutaneous edema, which were observed in post inoculation (p.i.) on day 3. This was when the highest embryonic mortality rate was recorded. From days 4 to 7 p.i., almost no embryonic mortality was observed. Therefore, the ELA could be optimized by reducing the observation time of the trials to 4 days p.i.. In conclusion, the ELA can be considered a reliable and useful tool to predict the pathogenicity of avian *E. faecalis* field strains by using the percentage of mortality. Proven high pathogenic isolates are candidates for autogenous vaccines.

Keywords: chicken, layer, *Enterococcus faecalis*, virulence, amyloid arthropathy

S3-0046 Survey of *Mycoplasma synoviae* prevalence in the Middle East and North Africa area

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The bacteria *M. synoviae* (MS) is a member of the mycoplasma genus. It causes disease in the joints, bones, respiratory tract and oviduct of birds. A MS infection can result in big economic losses due to a drop in egg production and eggshell quality. During last few years, it was noticed that some layer and breeder flocks faced a drop in egg production of ~10%, some after the peak, while other before reaching the peak of production, along with poor eggshell quality. Therefore, it was believed that it was valuable to investigate the prevalence of MS in the regions. Blood samples have been collected from the Middle East and North African area from flocks suffering from production drops, respiratory signs, and poor eggshell quality. Also, samples were taken from broilers showing respiratory problems. Blood samples were analyzed using serology-ELISA technique to detect the disease presence. Blood samples were collected from layers and breeders. At age < 16 weeks, 10% of the samples were found positive; While at age > 16 weeks, ~ 45% of the samples were found positive. Moreover, 4% of broiler samples were positive. From above, it was concluded that field challenge with MS was found during rearing and production in layers and breeders, in addition to its presence in broilers. The flocks were treated but the treatments were not sufficient to control the disease.

Keywords: *M. synoviae*, prevalence, Middle East, North Africa, production drop, eggshell quality

S3-0048 Impact of trace minerals on wound healing of footpad dermatitis in broilers

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Footpad dermatitis (FPD) can be used in the poultry industry as an animal welfare criterion and to determine stocking density. Trace minerals (TM) play a role in skin structural integrity and wound healing. This study evaluated the impact of TM on FPD, and consisted 3 treatments with the same basal diet and 3 supplemental TM levels: 0:0:0 ppm (WB0), 32:8:32 ppm (WB32), 64:16:64 ppm (WB64) of methionine hydroxy-analogue chelate of Zn:Cu:Mn (MINTREX[®], Novus International, Inc.). Fe, I, Se (40, 1.25, 0.3 ppm) were added to WB32 and WB64 but not to WB0. Each diet was fed to 9 replicate pens of 12 male broilers. Growth performance, FPD scores, and gene expression in footpad skin were measured. On d 21, 71% birds developed mild FPD and pens were top-dressed with dry litter to induce FPD healing. Compared to WB0, WB32 reduced ($P < 0.04$) FPD scores on d 28 and d 42, WB64 reduced FPD scores on d 43 ($P < 0.1$). Both WB32 and WB64 improved ($P < 0.03$) body weight (BW), gain, feed intake (FI) and performance index on d 14. WB32 improved ($P < 0.02$) cumulative FCR (cFCR) on d 28. WB64 improved ($p < 0.05$) BW, gain, FI on d 28 and cFCR on d 42. WB32 increased ($P < 0.04$) mRNA levels of MMP (matrix metalloproteinase) 13, TIMP (tissue inhibitor of metalloproteinase) 2, TIMP3, TIMP4, ITGA2 (integrin $\alpha 2$), ITGA3 (integrin $\alpha 3$), ITGB1 (integrin $\beta 1$) on d 15, MMP13 on d 43, and area under the curve (AUC) of CD40 and MMP13 during d 15-43. WB64 increased ($P < 0.04$) ITGA3 mRNA level on d 43. Both WB32 and WB64 increased ($P < 0.05$) mRNA levels of VEGF (vascular endothelial growth factor) on d 29, TIMP4 on d 36, CD40 on d 43, and AUC of TIMP2, TIMP3, TIMP4 and VEGF during d 15-43; decreased ($P < 0.02$) IL-1 β on d 22. TIMPs, MMP13, integrin $\alpha 2$, $\alpha 3$, $\beta 1$, and CD40 play key roles in collagen synthesis and organization, matrix remodeling and wound healing. VEGF stimulates angiogenesis. These results indicate that TM improved growth performance and promoted FPD wound healing in broilers.

Keywords: footpad dermatitis, lesion development, wound healing, trace minerals, gene expression

S3-0049 Monitoring the response of acute phase proteins pre and post vaccination after the administration of a combined Newcastle disease (ND) and infectious bronchitis (IB) vaccine in specific pathogen free (SPF) chicks, using commercially available ELISA kits

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Commercially available ELISA assays for AGP and SAA were validated by assessing their precision, accuracy, specificity and detection limit. The ELISAs used to monitor the levels of these APPs pre and post vaccination in 7 day old SPF chicks 80 after the intra-oculo administration of a combined live attenuated ND and IB vaccine (Treatment group). A saline was administered to 80 control SPF chicks. Blood samples were collected from a 6 chicks pre and post treatment at 12, 24 and then every 24 hours for a total of 6 days post treatment. The heterophil/lymphocyte (H/L) ratio were estimated each time point as a physiological stress indicator to determine a possible correlation between this ratio and APPs. To check that the vaccination had been successful, blood samples were collected at day 21 and 22 post treatment. A laboratory based ELISA was developed to confirm the presence or absence of antibodies to the vaccine in these samples. AGP increased at 24 hours in the treatment animals compared to the controls and was significantly higher at 2, 4 and 6 days post vaccination. SAA also increased in the treatment group but not until day 5, and only became significantly different on day 6. Antibodies to the vaccine were detected in the treatment group at day 21 and 22 post vaccination confirming that the vaccination protocol had been effective. The H/L ratio increased after one day post vaccination in the treatment group and remained significantly higher for 3 days post vaccination. This is consistent with the APP response detected. We conclude that AGP was the most sensitive to the administration of an industry standard combined ND and IB vaccination and that commercially available ELISA kits for this and other APPs could be useful in the early prediction of the efficiency of the immune response of these vaccines in commercial poultry. Moreover AGP could also serve as indicator to diagnosis of subclinical cases of ND & / or IB in chickens

Keywords: acute phase proteins, chicken, newcastle disease vaccine, infectious bronchitis vaccine, Alpha1- acid glycoprotein (AGP) , serum amyloid A (SAA)

S3- 0050 Effects of immunological stressors LPS and Poly(I:C) on physiology and performance traits of laying hens

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In order to study the effects of immunological stressors on laying hens, LPS and Poly(I:C) were injected in the vein of Rhode Island White hens under the wing at 53 weeks old. Then physiology and performance traits of the chicken were observed and measured. The result showed that the treatment of LPS could cause chicken drooping, lethargic, loss of appetite, diarrheal and fluffy feather. Compared to the untreated controls, the body temperature of the LPS treated hens declined 0.5°C at 3h post- injection ($P < 0.05$), the concentration of plasma IL- 6 decreased 17.57pg/mL at 8h ($P < 0.05$), the weekly average egg weight declined 2g ($P < 0.05$), and the laying rates of the first and second weeks post- injection declined 20.69% and 4.93% ($P < 0.05$), respectively. Moreover, hatchable egg rate and born weight of F1 generation significantly declined than the controls (decreased 8.96% and 0.97g, respectively) ($P < 0.05$). The treatment of Poly(I:C) significantly induced the increase of plasma TNF- α and IL-6 ($P < 0.05$). The concentration of IL- 6 was 18.94pg/mL and 15.76pg/mL higher than control group at 8h and 21d after Poly(I:C) treated, respectively. Egg passing rate and born weight of F1 generation declined 11.19% and 0.95g than the control hens, respectively ($P < 0.05$). In addition, Poly(I:C) could cause the loss of egg weight and body weight slightly. In conclusion, LPS and Poly(I:C) resulted in physiological function disorder and production performance decline of laying hens except they can induce immune-inflammatory response of the hens. LPS has worse effect than Poly(I:C).

Keywords: immunological stress, LPS, Poly(I:C), laying hens

S3-0051 *Eimeria* species in commercial broiler complexes in the united states

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To determine the prevalence of *Eimeria* species in commercial broiler complexes across the United States, oocysts were harvested from litter samples. DNA was extracted and species-specific primers were used to test for presence of *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. tenella*. Forty-three complexes from across the United States were sampled and species of *Eimeria* were determined using PCR diagnostics. The prevalence of each was as follows: *E. acervulina* (95%), *E. brunetti* (80%), *E. maxima* (87%), *E. mitis* (54%), *E. necatrix* (26%), *E. praecox* (85%), and *E. tenella* (77%). The seven mentioned species vary in pathogenicity, but have all been shown to cause decrease in performance, and in the case of *E. brunetti* and *E. necatrix*, mortality. The ubiquity of all of the *Eimeria* species warrants further investigation, as historically *E. acervulina*, *E. maxima* and *E. tenella* were the biggest concern for broiler farmers.

Keywords: *Eimeria*, coccidia, United States, PCR

S3- 0052 Preparation of effective monoclonal antibody against Gallus apoptosis inhibitor 5

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Apoptosis inhibitor 5 (API5) controls the G1/S cell cycle phase transition via E2F1. In this study, we firstly expressed the chicken API5 (chAPI5) protein in *Escherichia coli* (*E. coli*) and then produced two monoclonal antibodies (mAbs) against endogenous or recombinant API5 proteins. We cloned chicken API5 gene into prokaryotic expression vector pET-28a. Recombinant his fused API5 (His-chAPI5) expressed massively in supernatant when transforming the pET-28a-chAPI5 into *E. coli* (plyss). His-chAPI5 proteins were purified using NTA-agarose affinity resin and then used as immunogen to immunize female BALB/c mouse. Two hybridoma cells (MAb designated as 2D5 and 3D8) secreting MABs against His-chAPI5 protein were obtained through identifying antibody-producing cells by ELISA and Western blot assay. The ELISA, Western blot and IFA analysis indicated that these mAb specifically recognized His-chAPI5 protein, endogenous chAPI5 and 3flag-chAPI5 expressed in 293T cells. But IFA analysis indicated that the two mAb could not react with endogenous chAPI5 in DF-1 cell. Immunoglobulin subtype analysis showed that the heavy and light chain of the mAb was IgG1 and Kappa. Moreover, 2D5 seized endogenous chAPI5 protein as bait protein in the immunoprecipitation assay. These mAb against chicken API5 might be a useful tool in studying the function of chAPI5 protein in chicken cells.

Keywords: apoptosis inhibitor 5 (API5), monoclonal antibody, DF-1 cell

S3-0053 Avian paramyxovirus circulating in wild bird populations of the Azov-Black Sea region of Ukraine in 2006-2011

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Over the past 40 years many different paramyxoviruses have been isolated from animals and birds. APMV-1 and APMV-2, APMV-3, APMV-6, APMV-7 are important because they can cause disease in poultry. Objective. The goal of our research was to study APMV-1-9 circulating in different types of wild birds in Ukraine, by virus sequencing and examination of some of their biological characteristics. During 2006-2011, virological investigations were conducted on biological material from 5091 specimens collected from wild birds belonging to 52 species. Samples were collected in the Central and Eastern part of the Azov-Black Sea region of Ukraine. Twenty different serotypes of paramyxoviruses (APMV-1, APMV-4, APMV-6 and APMV-7) were isolated from the samples. Infection of wild birds of different species in different seasons was 0, 93-25,0%. APMV were mostly isolated from wild migratory waterfowl, except for APMV-4 which was isolated from starlings. The study of the biological properties of the APMV isolates showed different receptor specificity to erythrocytes of birds of 21 species and 4 species of mammals. Intracerebral pathogenicity index for all isolates was 0. Sequencing of most of the APMV-1 viruses determined they belonged to class II of NDV, and only one to class I. Most class II viruses belonged to I and II genotype, and only one to genotype XIV. A XIV virus genotype isolated from a white-fronted goose in 2011, has the cleavage site of protein F RRQKRF and is close to the strains which in 2008 resulted in a number of outbreaks in West and Central Africa. Conclusion. APMV-1 remain dangerous infection for poultry and require constant supervision and surveillance. Particularly noteworthy are the natural reservoir of the virus, wild birds that can carry pathogens over long distances, which can facilitate the introduction of dangerous viruses into new geographic regions.

Keywords: avian paramyxovirus, wild birds, monitoring, Azov-Black Sea region

S3-0054 Isolation and genetic characterization of avian influenza viruses from wild birds in the Azov-Black Sea region of Ukraine (2006-2012)

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Wild bird surveillance for avian Influenza virus (AIV) was conducted from 2006 to 2012 in Ukraine regions suspected of being intercontinental (North-South and East-West) flyways. A total of 6,281 samples were collected from wild birds representing 27 families and 11 orders. From these samples, 69 AIV's belonging to 15 of the 16 known hemagglutinin (HA) subtypes and 7 of 9 known neuraminidase (NA) subtypes were isolated. No H14 subtype was identified, as well as any N5 or N9 subtypes. In total, 9 H6, 8 H1, 9 H5, 7 H7, 6 H11, 6 H4, 5 H3, 5 H10, 4 H8, 3 H2, 3 H9, 1 H12, 1 H13, 1 H15, and 1 H16 HA subtypes were isolated. As for the N1 subtypes, 12 N2, 9 N6, 8 N8, 7 N7, 6 N3, 4 N4, and one not determined were isolated. There were 27 HA and NA antigen combinations. All viruses were low pathogenic AIV excluding 8 highly pathogenic AIV which were isolated during the H5N1 HPAI outbreaks of 2006-2008. Sequencing and phylogenetic analysis of the HA genes revealed epidemiological connections between the Azov-Black Sea regions and Europe, Russia, Mongolia and Southeast Asia. H1, H2, H3, H7, H8, H6, H9 and H13 AIV subtypes were closely related to European, Russian, Mongolian, and Georgian viruses. H10, H11, and H12 AIV subtypes were epidemiologically linked to viruses from Europe and Southeast Asia. Our results demonstrate the great genetic diversity of AIV's in wild birds in the Azov-Black Sea region, as well as the importance of this region for monitoring and studying the ecology of influenza viruses.

Keywords: avian influenza, wild birds, genetic characterization, Azov-Black Sea region of Ukraine

S3-0055 Effects of age and early or late sexual maturity on bone mechanical properties and egg production parameters of laying hens up to 40 weeks of age

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Osteopenia is a loss of bone mass due to osteomalacia and/or osteoporosis resulting in bone fragility and possible adverse effects on the performance, health and welfare of birds. This trial was conducted to investigate the relationships of bone mechanical properties with age and early and late sexual maturity of the hen up to 40 weeks of age. Two hundred fifty-six ISABROWN chicks were subjected to four different lighting regimens (8L:16D, 8L:16D increased to 14L:10D then reduced to 8L:16D, 11L:13D, 14L:10D) which were used to advance or retard sexual maturity. Birds were sequentially euthanized at 70, 91, 107, 119, 142, 158, 245 and 280 days and bone breaking strength (g and N) and tibial stiffness (N/deg) were assessed by using 3-point bending and torsional test. Egg production traits and shell quality during the laying period were also recorded. Tibial stiffness increased with age, especially during the early stages of the trial when the increase was steeper than the later stages, when a plateau was reached. A strong positive relationship between tibial breaking strength of all four groups, as assessed by both torsional and 3-point testing, and age was found and attained maximum levels when each group reached sexual maturity, but following sexual maturation of each group, tibial breaking strength started to decline slowly. Egg production of birds photoperiodically stimulated at 70 d (8-11L and 8-14L) was significantly higher than birds maintained on 8L, whereas birds with retarded sexual maturity (8-14-8L) had significantly lower egg production than the other treatments. As a result of late sexual maturity, birds on 8L continued to lay eggs with better shell quality than birds on 11L and 14L throughout the laying period. It was concluded that later maturing birds had stiffer and stronger bones and were able to withstand greater breaking forces than birds earlier maturing throughout the laying period and therefore the risk of broken bones might be reduced.

Keywords: osteopenia, bone strength, shell quality, laying hens

S3-0056 Transgenic Eimeria tenella expressing E. maxima profilin elicits enhanced protective immunity against wild type parasite infection in chickens

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Chicken coccidiosis is a widespread and economically significant disease caused by Apicomplexan parasites of the genus *Eimeria*. Vaccination in chicks with live parasites formulations has been considered the most powerful way to control this disease. However, successful vaccination of species of intermediate immunogenicity such as *E. tenella* relies on hosts' continuous intake of offspring oocysts which might cause reduced feed conversion limits the widespread application of anticoccidial vaccines in broilers. Apicomplexan parasites profilin has been tested as an immunodominant antigen and as for *Toxoplasma gondii*, a ligand of Toll-like receptor that initiates the hosts' innate immunity. In this study, we constructed 2 transgenic lines of *E. tenella* to evaluate the enhancement of immunogenicity of *E. tenella* by profilin. Et-EmPro expressed profilin of *E. maxima*, the highest immunogenic of 7 *Eimeria* species in chickens, and Et-TgPro expressed profilin of *T. gondii*. The output of oocysts of the transgenic lines and wild type parasite in chickens was measured daily between 4 and 14 days post immunization (dpi.), respectively. Challenge infection with wild type *E. tenella* in immunized birds was conducted at 14 dpi. and the total oocyst output was measured. We found there was an obvious peak of oocyst output at 10 dpi of Et-EmPro compared with Et-TgPro and wild type parasite immunized birds. Moreover, the oocyst output reduction rate was increased from 75% of wild type immunized chickens to 99% of Et-EmPro immunized. Our results demonstrated *E. maxima* profilin expressed by transgenic *E. tenella* enhanced the immunogenicity of *E. tenella* and vaccination with the transgenic line provided fully protective immunity against wild type parasite infection after single oral immunization. Our data encouraging Et-EmPro implemented as an alternative coccidiosis vaccine strain.

Keywords: transgenic *Eimeria tenella*, *E. maxima*, profilin, anticoccidial vaccine

S3- 0057 Molecular basis for the thermostability of Newcastle disease virus

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Thermostable Newcastle disease virus (NDV) vaccines have been used widely to protect village chickens against Newcastle disease, due to their decreased dependence on cold chain for transport and storage. However, the genetic basis underlying the NDV thermostability is poorly understood. In this study, we generated chimeric viruses by exchanging viral genes between the thermostable TS09-C strain and thermolabile LaSota strain using reverse genetics technology. Evaluations of these chimeric NDVs demonstrated that the thermostability of NDV was dependent on the origin of HN protein. Chimeras bearing the HN protein derived from thermostable virus exhibited a thermostable phenotype, and vice versa. Both hemagglutinin and neuraminidase activities of viruses bearing the TS09-C HN protein were more thermostable than those containing LaSota HN protein. Furthermore, the newly developed thermostable virus rLS-T-HN, encoding the TS09-C HN protein in LaSota backbone, induced significantly higher antibody response than the TS09-C virus, and conferred complete protection against virulent NDV challenge. Taken together, the data suggest that the HN protein of NDV is a crucial determinant of thermostability, and the HN gene from a thermostable NDV could be engineered into a thermolabile NDV vaccine strain for developing novel thermostable NDV vaccine.

Keywords: Newcastle disease virus; thermostable vaccine; HN protein; reverse genetics technology

S3-0058 Test report about influence of bioactive peptide DS- II on white feather broilers' production performance

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Test Objectives: For a long time, feeding antibiotics have played a significant role in the protection of animals' health, promoting animals' growth rate, improving feed utilization rate and have brought huge economic benefits on livestock and poultry production. However, with the extensive use of antibiotics in livestock and poultry, the problem of drug residues and drug resistance caused by antibiotics has been widely concerned. As the most promising new drug preparation to replace antibiotics, high efficiency, broad spectrum, and no drug residues of antimicrobial peptides has attracted the attention of researchers at home and abroad, and has become a hot research topic. This study has given eloquent proof that bioactive peptides DS- II played a significant role in strengthening the body immune system, repairing damaged tissue and improving broiler chickens' growth performance, and has provided further data bases on carrying out no-antibiotic cultivation. **Materials and Methods:** The study selected 16000 white feather broilers randomly divided into the control group and the experimental group as object and fed the trial group for 40 days. The survival rate, the feed-meat ratio, the average weight and the European index were recorded and analyzed. **Results and Discussion:** The results showed that the experimental group and the control group have kept consistent on the day of age and feeding density. The average survival rate of control group and experimental group were respectively 93.86%, 93.85%; the feed-meat ratio were respectively 1.68, 1.62; the average weight were respectively 2.27kg, 2.5kg; european index were respectively 323, 358. The test results showed that the bioactive peptide DS- II retained enormous influence on repairing body's gastrointestinal tract, effectively increasing the digestibility of the feeds, reducing the feed-meat ratio, improving average weight and promoting growth performance.

Keywords: antibiotics, drug resistance, antimicrobial peptides, no- antibiotic, growth performance

S3- 0059 Campylobacter in Central China: ST-1150 dominance and ST-464 strongest resistance related to its ability to form biofilm

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Poultry are recognized as a main reservoir of Campylobacter and consumption of poultry is considered to be an important source of human infection with Campylobacter, which lead to extensive spread antibiotics resistance. The objectives of this study were to determine the antimicrobial resistant profile, factors accounting for serious antibiotics resistance and dominant strains of Campylobacter isolates in Central China. 112 strains of bacteria have been isolated from 710 samples of anal swabs during 2013-2015 and its positive ratio is 15.8%. Among them, 106 strains (106/112, 94.64%) were Campylobacter jejuni (*C. jejuni.*) and 6 strains (6/112, 5.36%) were Campylobacter coli (*C. coli.*). Further, MLST typing results confirmed substantial genetic diversity as the 106 *C. jejuni* isolates generated 64 sequence types (STs). The ST-1150CC associated strains were the most dominant accounting for 25.9% (27/112). Whereas ST-828CC predominated accounting for of all *C. coli* isolates. Antibiotic susceptibility test demonstrated the analyzed isolates were all multidrug-resistant to more than five antimicrobials and most susceptible to aminoglycosides ($S \geq 78.6\%$), followed erythromycin ($S = 62.5\%$) and sulfamethoxazole ($S = 50.9\%$). Among all isolates, ST-464CC associated strains exhibited the strongest antibiotic resistance, whereas ST-21CC showed the least. The contributions of this work are therefore (i) Pathogenic ecology of this pathogen in Central China and clear association between lineage (clonal complexes) and phenotype (antibiotics resistance and biofilm formation) was elucidated. (ii) This work firstly reported ST-1150CC is the most dominant lineage and ST-464CC performance the strongest resistance in Central China, which is significantly related to their respective ability to form biofilm.

Keywords: Campylobacter, prevalence, antibiotic resistance, genotype lineage, biofilm

S3-0060 Inhibition of ERK / MAPK suppresses avian leukosis virus subgroup A and B replication

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We have previously shown that extracellular signal-regulated kinase / mitogen-activated protein kinase (ERK/MAPK) pathway contributes to subgroup J avian leukosis virus (ALV-J) replication and tumorigenicity. However, the role of ERK/MAPK pathway in the replication of subgroup A avian leukosis virus (ALV-A) and subgroup B avian leukosis virus (ALV-B) remains unclear. In this study we successfully constructed and recovered ALV-A strain GD13-1 which showed similarities in growth to the parental wild type virus in vitro. And we observed that ALV subgroup J, A or B could trigger ERK2 activation in CEFs. Inhibition of ERK / MAPK markedly suppresses ALV-A and ALV-B replication, as evidenced by extremely low levels of viral transcription and virus protein production. This finding provides evidence that ERK/MAPK signaling responses play important roles in ALV replication and may represent novel drug targets for therapeutic intervention strategies.

Keywords: ALV-A, ALV-B, ERK/MAPK pathway, virus replication

S3-0061 Progeny serum biochemical profile among different age groups and close bred flocks using different selection strategies

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The objectives of commercial poultry breeding are the upgrading of production potential and eradication of disease threats. Present study evaluated serum biochemistry in progeny as influenced by different age groups and close bred flocks using different selection strategies. A total of 540 quail breeders were subjected three selection strategies having 4 close bred flocks and 3 age groups. In serum biochemistry, progeny of pedigree base selected birds had higher albumin/globulin ratio, while the progeny of mass selected birds had higher serum total protein, albumin and glucose, where- as the progeny of random bred control group had higher serum globulin. Higher protein level in progeny of mass and pedigree selected birds could be attributed to their growth rate as compared to random bred control. Significant increase in serum total protein, albumin, A/G ratio and glucose were observed in progeny of selected parents as compared to random bred control. Progeny of mass base selected birds had higher serum glucose level followed by pedigree base selected and random bred control birds. These results highlight the importance of blood parameters in poultry industry.

Keywords: quail, selections strategies, serum biochemistry, close bred-flocks, age groups

S3- 0062 ALV- J infection induces antiviral innate immune responses of chicks and chicken with neoplasm in vivo

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Avian leukosis virus subgroup J (ALV- J) infection can cause tumors and immunosuppression. Since the precise mechanism of innate immune response induced by ALV-J was still unknown, we investigated the antiviral innate immune responses induced by ALV-J in chicks and chicken with tumor. In this study, the secretion of interleukin-6 (IL-6), IL-10, IL-1 β and interferon- β (IFN- β) were not significantly different between the infected chick group and the control group from 1d post hatch to 7 d post hatch. Nevertheless, IL-6, IL-1 β and IFN- β protein expressions in the three clinical samples with neoplasm dramatically increased compared to the healthy samples. It was noticeable that IL-10, as the anti- inflammation cytokine, was also increased sharply in the two of three clinical samples. We found more than 20-fold up-regulation of ISG12-1 mRNA at 1 d post infection (1DPI), and greater than 2-fold up-regulation of ZC3HAV1 mRNA at 4DPI. However, there was no statistical difference in ISG12-1 and ZC3HAV1 mRNA expression in tumorigenesis phase. Besides, ALV-J mainly induced significant increase of Toll-like receptors 7 (TLR-7) at 1DPI and dramatically increased the mRNA expression of melanoma differentiation- associated gene 5 (MDA5) in tumorigenesis phase. Moreover, the protein levels of interferon regulatory factor 1 (IRF-1) and signal transducer and activator of transcription 1 (STAT1) decreased in the chicken with tumor. These results suggest that ALV-J was primarily recognized by chicken TLR7 and MDA5 respectively at the early stage of infection and late phase of infection in vivo. ALV-J strain SCAU-HN06 can hardly induce antiviral innate immune response in one week old chicks. However, interferon-stimulated gene (ISG) expression can't be induced normally during the late phase of ALV-J infection due to the reduction of IRF1 and STAT1 expression.

Keywords: ALV- J, cytokines, innate immune response

S3-0063 Comparative study of three infectious bursal disease (IBD) intermediate plus vaccines in broiler chickens

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The objective of this study was to compare ELISA titer development, immunosuppressive effects and bursal lesions of three IBD intermediate plus vaccine strains. Three groups of commercial broilers were individually identified and housed separately. At 13 days of age the three groups were vaccinated orally by crop instillation with the strain V217 (AviPro® IBD Xtreme), GM97 (Hipragumboro® GM97) and V877 (Poulvac® Bursa) (groups 1, 2 and 3 respectively). All treatment groups were vaccinated at Optimal Day of Vaccination calculated with Deventer Formula according to breakthrough titer of 600. At 27 days of age, all groups received via eye drop a live ND vaccine. Blood samples (20 per group) were collected at IBD vaccination day and at 7-day intervals (7, 14, 21, 28 and 35 days) post IBD vaccination. All the samples were tested for IBD specific antibodies (BioChek® ELISA kits). On day of ND vaccination and at 7, 14 and 21 days post ND vaccination the sera were tested for ND specific antibodies by Haemagglutination Inhibition (HI) test. At 48 days of age, from five birds per group bursas were collected for RT-PCR, VP2 gene sequencing, histological examination and scoring. The IBD vaccine strains were detected in the bursal tissue of respective treatment groups and the histopathological analysis revealed that the lowest bursal lesion scorings were in groups 1 and 2 (3.6). At 21 days post ND vaccination, group 1 obtained a ND mean antibody titer significantly higher ($P<0.05$) in comparison to groups 2 and 3. Regarding the IBD antibody response, group 1 presented higher serological IBD mean titer than groups 2 and 3 at 7, 14, 21, 28 and 35 days post IBD vaccination. At 27 days of age group 1 had significantly ($P<0.05$) higher IBD mean titer in comparison to groups 2 and 3. Although all the IBD intermediate plus vaccine strains elicited a serological response, the V217 strain demonstrated higher immunogenicity than the other two strains.

Keywords: Gumboro, vaccination, intermediate plus, protection

S3-0064 Effects of coccidia infection on broiler genotypes divergently selected for performance traits

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144 male day old chicks each of a fast growing (F) and a slow growing (S) line were used to test the hypothesis that genetic selection for increased performance increases susceptibility to coccidiosis in a 2 x 3 factorial experiment. F and S birds were orally inoculated at 2 weeks of age with water containing 0 (C), 2.5 (L), or 7.0×10^3 (H) sporulated *Eimeria maxima* oocysts. Treatment groups were replicated in 8 pens with 6 birds per pen. Average daily gain (ADG), feed intake (ADFI) and feed conversion ratio (FCR) were measured post infection (pi) over the pre-patent (d0-d4), acute (d5-d8) and recovery (d9-d12) periods of infection. Plasma levels of α -tocopherol and retinol, and intestinal lesion (IL) scoring were measured at d6 and d13 pi. ADG and ADFI were expressed as a proportion of body weight at the start of infection. Genotype affected ($P<0.0001$) ADG and FCR, being higher and lower respectively for the F than the S genotype. Dose affected ($P<0.0001$) ADG, ADFI being higher, and FCR being lower for C than L or H birds. Period ($P<0.0001$) and its interaction with dose ($P<0.0001$) affected ADG, FCR and ADFI ($P<0.0001$), as infection reduced performance during the acute period. Genotype affected both FCR and ADG ($P<0.0001$) being lower and higher respectively for the F genotype. Genotype and dose did not interact for any of the measured performance variables. Only dose affected IL and plasma levels of α -tocopherol and retinol at d6. Infection induced IL in infected birds and reduced plasma levels of retinol and α -tocopherol ($p<0.0001$). At d13 pi no IL were detected, whilst α -tocopherol level was still significantly lower ($p<0.05$) and retinol tended to be higher ($p<0.1$) in infected than C birds. Results indicate that genotypes divergently selected for performance did not differ in their resistance or tolerance to *E. maxima* infection when offered high quality diets.

Keywords: broiler, performance, coccidia infection, genetic selection

S3-0065 Gene detection, virus isolation, and sequence analysis of avian leukosis viruses in eurasian tree sparrows in China

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To understand the current status of avian leukosis virus (ALV) infection of eurasian tree sparrow (*Passer montanus*). We collected 160 samples from different regions in China. Virus isolation and PCR amplification showed that some of the eurasian tree sparrows were infected with ALV, and 6 strains ALV-A were isolated from the samples. The gp85 gene from each of the 6 ALV-A strains was amplified, cloned, and sequenced. Sequence analysis indicated that the gp85 genes of the 6 ALV-A strains have the highest homology with SDAU09C3 isolated from layer chickens, 98.2%, and 97.8% homology with American strains MAV-1. These results demonstrate the presence of ALV-A in eurasian tree sparrow and may be come from layer chickens, and the results will not only provide necessary information for further understanding the evolution of ALV, but they also identify the potential role of wild birds in ALV transmission and furthers understanding of the ecology of ALV in wild bird species.

Keywords: Eurasian tree sparrow, avian leukosis virus (ALV), sequence analysis

S3-0066 Ease vaccination program with the use of next generation HVT recombinant vaccines, improving bird health and performance

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Around 2003 first generation of HVT recombinant vaccines entered the markets. The first generation HVT recombinants (rHVT) are based on HVT as vector with 1 insert from different viruses like ND, IBD, ILT or AI. HVT is practical vector because as herpes virus it gives repeated immunity induction resulting in a long duration of immunity without reaction, besides there is room in the genome for inserts and can be given as Marek's vaccine. rHVT recombinants have a number of nice characteristics that can improve birds welfare and ease the vaccination program. The rHVT-recombinant offers combined control of 2 diseases in one shot (2 in 1 -Marek + insert), avoid vaccination reaction from live full virus vaccine, move more basic vaccination towards the hatchery and create space in the vaccination program for other vaccinations like IBV. The hatchery is a place where vaccine application can be standardized with the use (semi) automatic administration equipment (semi automatic Marek injectors for use at day-old and In ovo injection used on an 18 days old embryo). The use of standardized equipment will reduce the possible human influence on accuracy of injection. The first generation rHVT-ND, rHVT-IBD, rHVT-ILT and rHVT-AI are used more and more every year around the globe. In the meantime R&D groups worked on the next generation of recombinant HVT where they put 2 inserts in the vector to create a 3 in 1 vaccine, one shot inducing control against 3 diseases. In this paper we will discuss the development, on safety and efficacy of a new HVT recombinant the rHVT-ND-ILT, based on a construct of rHVT-F-gI-gD. The HVT genome is carrying the genetic material from the F-protein of ND and gI and gD proteins from ILT. Data show that the next generation rHVT-ND-ILT, is safe and efficacious.

Keywords: HVT recombinant, hatchery vaccination, vaccine

S3-0067 Genetic and antigenic characteristics of H9N2 chicken influenza viruses isolated in Northern China from 2014 to 2015

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We previously demonstrated that genotype G57 of H9N2 avian influenza virus (AIVs) became predominant in Chicken since 2010 and antigenic variants isolated during 2009-2013 formed a novel HI antigenic group F. To understand the genetic and antigenic characteristics of prevailing H9N2 AIVs in northern China, we performed genetic and antigenic characterization using H9N2 viruses isolated from chickens during 2014-2015. Genetic analyses revealed that all the isolates belonged to genotype G57 in addition to a strain (A/Chicken/Beijing/XY1206/2014). The nucleotide homologies of hemagglutinin (HA) genes ranged from 95.3 to 99.9%. Compared with the vaccine strain (A/chicken/Shanghai/F/1998), most HA of the tested viruses lost the potential N-linked glycosylation site (PGS) at residues 127-129 and 200-202, but obtained another PGS at residues 64-66. Antigenic analyses by conducting hemagglutination inhibition and neutralization assays using a panel of polyclonal antibodies confirmed the antigenic drift of recent H9N2 viruses. Most of these 2014 isolates belonged to HI antigenic group F, while all the isolates of 2015 formed a novel HI antigenic group G, which might be result from 7 mutations predominant in the 2015 viruses. Taken together, our results indicate that H9N2 AIVs of 2015 form a novel HI antigenic group G in northern China. It is necessary to take systematically surveillance of H9N2 AIVs and timely update vaccine strains in China.

Keywords: H9N2, avian influenza virus, genetic evolution, antigenic changes

S3-0068 Genetic evolution of H9N2 influenza viruses isolated from China during 1994-2013

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In China, H9N2 influenza virus has undergone extensive genetic evolution since first isolation in chickens in 1994. However, a systematic phylogenetic analysis has not been undertaken since 2008. Here, we collected all available published data including genomic sequences of 732 H9N2 strains isolated in China from 1994 to 2013 and performed phylogenetic and genotypic analysis. The 732 H9N2 viral genomes were classified into 62 separate lineages and panorama genotypical analysis revealed that H9N2 viruses included at least 117 genotypes. From 1994 to 1999, G01, G03 and G11, belonging to previously identified series BJ94, were the dominant genotypes sporadically distributed in mainland China. At the year of 2000, the internal segments of aquatic bird and duck lineages originated from Korea were introduced into China and led to a dramatic increase of genetic diversity with at least eighty genotypes emerging in following five years. During that period, G38 and G42 were the predominant genotypes, and Guangdong province, located in south China, was the major epidemic focus. Minor avian species were the primary generator and disseminator of G38 and G42 H9N2 viruses. Since 2006, the number of genotypes was sharply decreased into 33 genotypes and all of the major genotypes belonged to previously identified F98 series which is generated in chickens. After the year of 2010, G97 gradually became the only major genotype which seems emerged in eastern China and transmit to the regions throughout China. This genotype contributes its six internal segments to over ten novel reassortants including H7N9 and H10N8 influenza virus. Compared with minor poultry, chicken plays more important role recently to generate new genotypes and transmit them across the species barriers. Taken together, our results present a comprehensive understanding on spatio-temporal genetic evolution of H9N2 influenza viruses in diversified hosts from 1994 to 2013 in China.

Keywords: H9N2, influenza virus, genetic evolution, host

S3- 0069 Rapid detection of clade 2.1.3 H5N1 influenza viruses using a pyrosequencing method

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Highly pathogenic H5N1 avian influenza viruses have caused 844 confirmed human infections in 15 countries. Among these countries, Indonesia has the highest case number and a significantly higher mortality rate than other countries. Statistical studies indicated that clade 2.1.3 viruses are predominant in causing people infections in Indonesia, which highlights the significance for developing a rapid and robust method to identify clade 2.1.3 H5N1 influenza viruses. Phylogenetic analysis of the hemagglutinin (HA) gene suggested that different clades of H5N1 viruses shared high homology, which is hard to be distinguished using conventional RT-PCR detection method. Analysis of HA cleavage site sequences from H5N1 viruses available in databases revealed that the P6 position of the cleavage site was the most polymorphic (G, I, R, *, S) and 88.7% of clade 2.1.3 viruses contained a serine at the P6 position. In this study, we developed a rapid pyrosequencing detection method for clade 2.1.3 H5N1 viruses. Primers were designed based on the residue at the P6 cleavage position in HA to identify the unique serine of clade 2.1.3 viruses. The forward primer was biotinylated to amplify a 170bp fragment containing target sequences. Sequencing primer used for pyrosequencing was designed to identify a 50 bp region covering the cleavage site. Pyrosequencing reactions were performed for viruses containing 325G, 325I, 325R, 325*, and 325S. Results showed that the residue at P6 cleavage position was correct in all the test viruses and no cross-reaction with other subtypes of influenza viruses. A total of 423 clinical specimens collected in China were tested and no sample was found to contain the serine at P6 cleavage position. Collectively, our study demonstrated that the pyrosequencing assay is a specific, sensitive and reliable method suitable for rapid identification of clade 2.1.3 H5N1 avian influenza viruses.

Keywords: clade 2.1.3, H5N1, HA, pyrosequencing

S3- 0070 Prevailing mutations in PB2 protein contribute to increased infectivity of avian H9N2 influenza virus in mice

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H9N2 avian influenza viruses (AIVs) have occasionally crossed species barrier to infect mammals during their prevalence in Eurasia. Recently, several studies have indicated an increasing mammalian adaption of prevalent H9N2 AIVs. H9N2 AIVs are also considered as the gene donor of novel H7N9 and H10N8 AIVs that cause human infection and death. To investigate the genetic basis of H9N2 AIVs' host specificity, we carried out comprehensive phylogenetic analyses for PB2 gene, using all available sequences. We found that the prevailing PB2 gene carried PB2- I292V/T598V/L648V mutations. H9N2 AIVs with the mutations I292V and T598V replicated more efficiently than wild viruses in human A549 cells and mice, and enhanced polymerase activity in human 293T cells. In addition, PB2-I292V inhibited the expression of type I interferon on mammalian cells, which may correlate with higher replication of viruses in mice. In summary, our results suggested that the prevailing mutations PB2-I292V and PB2-T598V contributed to increased infectivity of avian H9N2 influenza viruses in mice.

Keywords: H9N2, mutations, PB2, infectivity

S3- 0071 Increased pathogenicity of H9N2 avian influenza viruses isolated in China, 2015

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H9N2 influenza virus has low pathogenicity for avians, replicating mainly in the upper respiratory tract and causing mild or no overt signs of illness in chickens. However, several researches have shown that the H9N2 influenza viruses isolated from China after 2010 displayed high virulence to chickens. Here, researches have been conducted in specific pathogen-free chickens to understand the pathogenicity of H9N2 influenza viruses which were isolated from poultries in China from 1999 to 2015. Histologically, the epidemic isolates in 2015 caused necrosis of trachea epithelium cells, bronchopneumonia, necrosis of pancreas, mild necrosis of spleen and renal tubular epithelial cell degeneration. These viruses replicated well in the trachea and lung, and the virus titer level was significantly higher than that of the earlier viruses ($P<0.05$). Moreover, the viruses isolated in 2015 were detected in liver, spleen, kidney, bursa and thymus, indicating systemic infection. In addition, most of the viruses in 2015 clearly showed enhanced virus shedding in the cloaca compared with those of earlier isolates. By detecting the virus in blood, we found that the severe disease caused by recent viruses was associated with early viremia, which suggests that viremia is the key to dissemination of H9N2. Our findings demonstrate that the H9N2 influenza viruses isolated from poultries in China from 1999 through 2015 are becoming progressively more pathogenic for chickens, which may cause dramatic economic loss to poultry industry and increase the possibility of H9N2 viruses in reassortment with other subtype viruses.

Keywords: H9N2 avian influenza virus, chicken, pathogenicity, viremia

S3- 0072 A novel adjuvant for cell mediated immune response improves protection conferred by avian influenza poultry vaccines

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Water-in-mineral oil adjuvants induce a strong long-term humoral immune response and are widely used in poultry vaccines. New adjuvants that also increase the cellular immune response could help to extend the vaccinal cross-protection against viral strains variants. Here we demonstrate that the water-in-oil adjuvant Montanide™ ISA 71 VG (ISA 71) developed to stimulate both humoral and cell mediated immune responses improves vaccine efficacy in poultry. In a first trial, 10 chickens per group were injected with experimental Newcastle Disease and H9N2 Avian Influenza (AI) vaccine adjuvanted with standard water in oil adjuvant or with ISA 71. Vaccine efficacy was assessed by ELISA antibody titration up to D42. In a second trial, ISA 71 was tested in an inactivated H5N1 AI vaccine model. The safety of the vaccine was first assessed by injection of 2ml dose in SPF chickens. Efficacy profile of the adjuvant was then assessed in SPF chickens and in 7 days old broiler chickens. Chickens were injected with experimental AI vaccines adjuvanted with standard water in oil adjuvant, with aluminium hydroxide or with ISA 71. Blood samples were taken from non-challenged chickens up to 6 weeks post vaccination for antibody titration. 10 chickens of each group were challenged with AI virus subtype H5 at D14 post vaccination. In the first trial, ISA 71 vaccine induced significantly higher antibody levels than other formulations from D0 to D28. The use of ISA 71 also allowed a reduction of the antigenic load to 25% of the original concentration. In the second trial, ISA 71 vaccine induced significantly higher antibody levels than other formulations in SPF and broiler chickens. The ISA 71 formulation was also the only vaccine able to reach 100% protective levels after challenge at D14 in broiler chickens. We could demonstrate that Montanide™ ISA 71 VG is safe and is an efficient adjuvant for avian influenza vaccine formulation.

Keywords: vaccine, avian influenza, adjuvant, Montanide

S3-0073 A robust and flexible adjuvant formulation for potent and stable poultry vaccines

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Water-in-mineral oil emulsion adjuvants induce a strong long-term immune response and are widely used in inactivated poultry vaccines. As chickens receive multiple vaccines, multivalent vaccines are more and more used in the field to decrease the number of injections. Resistant adjuvant formulations are therefore needed to ensure stability of vaccines, especially for multivalent bacterial vaccines. We have thus developed a new adjuvant resistant to destabilizing antigenic media and conditions, and that can be used at flexible ratio in poultry vaccines. First, vaccines based on model antigenic medium were prepared by using Montanide[™] standard and Montanide[™] ISA 71R VG (ISA 71R) resisting adjuvant for poultry vaccines and were compared for emulsion stability over time. Then, safety and efficacy properties of resisting adjuvant were tested in chicken using a *Pasteurella multocida* antigen using 60% of adjuvant in the vaccine. In a second trial, a trivalent infectious coryza (serovars A, B and C) vaccine was formulated with ISA 71R and tested in chicken. An infectious challenge was realized for each valence of the vaccine. We could show that the resistant adjuvant ISA 71R allows the formulation of stable vaccines able to pass severe stress tests. Formulations based on ISA 71R showed comparable acceptable safety levels and efficacy profiles than reference vaccine formulations in chicken trials. Moreover, the vaccine based on ISA 71R induced full protection against the 3 tested infectious coryza strains. This study shows that Montanide[™] ISA 71R VG can be used at flexible ratio and is able to resist destabilizing antigenic media. This adjuvant also induces high antibody levels and has an acceptable safety profile in poultry, even combined with reactogenic Gram negative bacterial antigens. Such an adjuvant can allow the development of stable and efficient multivalent vaccines for which long term emulsion stability is correlated with potency stability.

Keywords: vaccine, adjuvant, coryza, *Pasteurella*, Montanide

S3-0074 Genetic characterization of fowl adenovirus serotype 4 isolated from chickens associated with inclusion body hepatitis and hydropericardium syndrome in China, 2015

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In 2015, severe outbreaks of chicken inclusion body hepatitis (IBH) and hydropericardium syndrome (HPS) have re-emerged in China, causing considerable economic losses. To identify and characterize the causative agent of the disease, 195 samples from livers of IBH/HPS-affected birds were collected from five provinces of China. The samples were identified for fowl adenovirus (FAdV) by a hexon-based polymerase chain reaction combined with restriction enzyme analysis of the amplified DNA fragments. The complete genome of one FAdV isolate from each province was determined by using the Sanger dideoxy sequencing method. The complete genome sequences of the five FAdV isolates were aligned with other available FAdV genome sequences in the GenBank database to determine the nucleotide sequence homologies using the ClustalW multiple alignment algorithm in the MegAlign program of the DNASTar software. Phylogenetic trees for the complete genome, peton, fiber, and hexon genes were constructed by using the maximum-likelihood method in MEGA 6.06 software. The results showed that all the samples were FAdV positive, and identified as FAdV serotype 4 (FAdV-4). The full genome for five isolates was found to be 43,721-43,725 bp in length, respectively. All five isolates shared high complete genome and structural gene nucleotide identities of 99.9% - 100%, and were clustered into one group different from earlier reported FAdV4 strains. When compared with earlier reported strains MX-SHP95, KR5, ON1, nucleotide identities of 98.8%, 98.4%, 98.0% were seen for the complete genomes, 95.8%, 96.7%, 95.7% for fiber 1, 97.2%, 95.9%, 95.8% for fiber 2, 98.6%, 98.9%, 98.7% for hexon, 99.2%, 99.0%, 98.9% for peton, respectively. This study demonstrated that the outbreaks of IBH/HPS in China were primarily caused by variant FAdV4, suggesting that preventive measures against FAdV4 infection on poultry farms should be implemented in China.

Keywords: fowl adenovirus serotype 4, variant, chicken inclusion body hepatitis, hydropericardium syndrome

S3-0075 Evaluating relationships between intestinal health and broiler chicken performance using a global health dataset

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Intestinal health plays a vital role in reducing environmental impact and optimizing health, welfare, and food safety. Our objectives were to: 1) investigate risk factors for: average daily gain (ADG), feed conversion rate (FCR), European Production Efficiency Factor (EPEF), and week one % mortality; these reflect production efficiency and impact environmental and economic sustainability, and 2) to investigate the relationship between the Intestinal Integrity (I2) index and these performance measures. Data were obtained from Elanco's Health Tracking System (HTSi), a global poultry health surveillance system in which 23 intestinal conditions are recorded from standardized necropsies; these are used to evaluate the cumulative impact of enteric disease by calculating the I2 index. Approximately 3,500 flock-level health observations (2006-2015) were integrated with I2 scores and performance and management data for analysis using multivariable generalized linear models to identify risk factors for production measures and associations with I2. Excessive intestinal fluid, gizzard erosions, gross E. maxima, and days out were negatively associated with ADG; excessive intestinal fluid, gross E. acervulina, gizzard erosions, roundworms, and feed passage were positively associated with FCR; excessive intestinal fluid, gross E. acervulina, and gizzard erosion were negatively associated with EPEF; and microscopic E. maxima, necrotic enteritis, thin intestines, and excessive intestinal fluid were positively associated with % mortality in week one ($P < 0.05$ in each case). As I2 increased by one unit, ADG increased by 0.04 g ($P = 0.001$), FCR decreased by 0.0013 points ($P < 0.001$), and EPEF increased by 0.52 ($P < 0.01$). This demonstrates that intestinal health has a substantial impact on performance, and hence profitability and sustainability. The I2 index is an important tool that allows producers to monitor, benchmark, and inform and evaluate changes to improve intestinal health.

Keywords: chicken, benchmarking, intestinal health, surveillance, performance

S3- 0076 DPS antibacterial peptide used in broilers' technology demonstration of ecological farming

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China is in the evolution process from traditional animal husbandry transition to modern animal husbandry, breeding scale is continually expanding, collective contents of manufacture is continuously improving, but accompanied is the complex diseases of poultry and livestock, the phenomenon using blindly antibiotic. Since the discovery of penicillin in 1928, antibiotics have saved tens of thousands of lives, and promoted the rapid development of the breeding industry, but the emergence of bacterial resistance is reversing the miracle of the past 80 years. In the course of breeding, exploring the alternative of antibiotics and chemicals those are efficient, no-residue has become current research hot spots. The study has compared differences in broilers' physiological performance and growth performance under the condition of no-antibiotics and adding DPS antimicrobial peptide, provided data for broilers' healthy farming practices and standards. The study selected 8 sheds broilers randomly divided into control group and experimental group after common arrangement. In the experimental group, the peptides were used to substitute the antibiotics, and the control group was in accordance with the existing health care administration procedures for 40 days. Survival rate, feed intake, feed-meat ratio, average weight and European index were recorded and analyzed. The results showed that average survival rate of control group and experimental group were respectively 91.53%, 94.66%; feed intake were respectively 3.97kg, 4.10kg; feed-meat ratio were respectively 1.63, 1.58; average weight were respectively 2.35kg, 2.57kg; european index were respectively 318, 349. The test results showed that DPS antibacterial peptide could reduce the mortality of broilers, effectively increase feed intake and feed conversion rate, reduce the ratio of feed to meat, improve the average weight, improve the growth performance.

Keywords: DPS antimicrobial peptide; antibiotics; ecological breeding

S3-0077 The papain-like protease of infectious bronchitis virus has deubiquitinating activity

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Structural and enzymatic studies have revealed that coronavirus papain-like proteases (PLPs) can act as both a protease to process virus encoded large replicase polyproteins and also as a deubiquitinating (DUB) enzyme to cleave the isopeptide bonds found in polyubiquitin chains. Furthermore, the viral protease/DUB activity can modulate or block activation of the innate immune response pathway utilizing different mechanism. For example, PLP2 of mouse hepatitis virus A59 (MHV-A59) can bind to IRF3, cause its deubiquitination, as a consequence, strongly inhibits IRF3 mediated IFN- β activities. HCoV-NL63 PLP2 can bind to RIG-I and ERIS, cause theirs deubiquitination, and reduce IFN induction. SARS PLP can remove ubiquitin from molecules that play key signaling roles in the induction of interferon, such as RIG-I, TRAF3 and ERIS. Kong, L.Y. et al has found that IBV PLP catalytic core domain expressed in *Escherichia coli* can degrade both K-48 and K-63 linked polyubiquitin chains to mono-ubiquitin. Here, we report the DUB activity of IBV PLP in virus infected DF1 cells and the identification of core domain of DUB activity. Firstly, pRK5- HA-Ub, pRK5-HA-K48, pRK5- HA-K63 were transfected into IBV infected DF1 cells respectively, and the DUB activity was analyzed by western-blot. The result showed that IBV can perform DUB activity in DF1 cell, especially at the early period of IBV infection. To further identify the core DUB functional domains of IBV PLpro, eukaryotic expression plasmids, PLP and PLP-TM (contained C-terminal transmembrane domain), were constructed and co-transfected with pRK5- HA-Ub, pRK5-HA-K48 or pRK5- HA-K63 into DF1 cells respectively. The result revealed that PLP-TM but not the PLP perform DUB activity in co-transfected DF1 cell. These results indicated that IBV may utilize similar strategy to antagonise host innate antiviral responses. and this nature could be applicable to develop strategies targeting PLP for the effective control of IBV infection.

Keywords: infectious bronchitis virus, papain-like protease, deubiquitinating activity

S3-0078 Bidirectional regulation of AvBD6 on the peripheral blood lymphocytes proliferation

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In order to explore the effect of avian beta-defensins 6(AvBD6) on chicken breed Roman Brown peripheral blood lymphocytes (chPBLs) under different condition, the chPBLs proliferation was analyzed by MTT method. Lipopolysaccharide (LPS) and adrenocorticotrophin (ACTH) were used imitated endotoxin and immunosuppression respectively. AvBD6 show different effect on the ChPBLs proliferation at different status. ACTH (5.5 μ M~690 μ M) inhibited ChPBLs proliferation significantly, and with a dose-dependent manner at 172.5~690 μ M. AvBD6 has no significant effect on ChPBLs proliferation at 4~100 μ M, but it inhibit ChPBLs proliferation at 200~800 μ M. AvBD6 (4 μ M) can synergistic stimulate ChPBLs proliferation with LPS at 0.031~0.5 μ g/mL, and AvBD6 (5~20 μ M) can do well with LPS at 1 μ g/mL. The minimum concentration of AvBD6 to active the ChPBLs proliferation suppressed by ACTH at 3.5 μ M and 7.0 μ M, was 25 μ M and 50 μ M respectively. The sequence of linear B- cell epitope of MC2R overlaps with MC5R but not TLR. Antiserum against TSAVHQ (anti- TSAVHQ) was used to block MC2R and MC5R. With a dose-dependent manner, anti- TSAVHQ with the concentration of 1/2~1/8 stock solution can block the inhibition of ChPBLs proliferation by ACTH at 172.5 μ M. The anti-TSAVHQ with the concentration of 1/2 and 1/4 stock solution can block the inhibition of ChPBLs proliferation by AvBD6 at 320 μ M. We can initially speculate that, at early stage of the gram-negative bacteria infection with low level of LPS and AvBD6, AvBD6 can activate the ChPBLs proliferation and show pro-inflammation with LPS; rather late in infection with high level of LPS and AvBD6, AvBD6 may inhibit ChPBLs proliferation and show anti-inflammation; AvBD6 can activate the suppressed ChPBLs proliferation by stress hormone ACTH. Therefore, AvBD6 may be a new molecule to maintain the immune homeostasis of chicken, and MCRs may be the co-receptor in the combination of AvDB6 or/and ACTH with ChPBLs.

Keywords: avian beta-defensin, lipopolysaccharide, ACTH, melanocortin receptors, chicken peripheral blood lymphocytes

S3-0079 Detection of avian influenza virus from fecal samples by quantitative realtime RT-PCR

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Avian influenza virus (AIV) is one of the most infectious diseases that occur in birds, and if not well managed can infect humans. AIV is from the family Orthomyxoviridae and the genus influenza virus A. The level of the viral infection on the bird host depends on the strain of the virus. The strain can have a low or high pathogenicity, depending on its antigenic hemagglutinating and neuraminidase activities on their lipid bilayer surface. As the virus evolves, new strains of the virus evolve over time, which increases the rate of its pathogenicity. The concern remains on the challenge to be able to detect the viral load of AIV in birds. We hypothesize that using real-time PCR (qPCR) to quantify the viral extent on the fecal samples will help in detection of the total viral count of AIV in birds. The Ribonucleic acid (RNA) was extracted from the fecal sample using TRIzol LS Reagent. The reverse transcription was carried out to make cDNA. Finally, qPCR was carried out to quantify the total viral count. The total viral load of AIV present in the fecal samples of the wild birds was quantified. In conclusion, we were able to detect the concentration of Avian Influenza virus in the fecal samples using qPCR. This technology will shorten the time of AIV detection.

Keywords: avian influenza virus, qRT-PCR, fecal samples, detection

S3-0080 Broiler coccidiosis vaccination: performance of flocks using vaccine alone or as part of an ionophore or chemical shuttle program

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Broiler coccidiosis has traditionally been controlled by in-feed ionophore or chemical anticoccidials. To maintain the efficacy of these products, producers have used product rotation strategies (different products used in successive flock cycles) and shuttle programs (different products used within a single flock cycle). Live coccidiosis vaccines have gained popularity within several countries. Two countries with coccidiosis vaccination used in 15% - 25% of annual broiler production are Argentina and the US. The strategies used by these countries for successful coccidiosis vaccination include seasonal rotation of coccidiosis vaccine with in-feed anticoccidial products as well as shuttle programs that involve the use of vaccination at the hatchery followed by an anticoccidial shuttle program within the same flock. Success of the vaccine-anticoccidial shuttle is highly dependent upon the timing and duration of the introduction of the anticoccidial. Regular post-mortem sessions at integrators using different coccidiosis vaccination strategies provide a database that can be used to compare programs. Lesion scores at various time points within flocks demonstrate the normal pattern of coccidiosis lesions following vaccination and the alteration of those patterns when anticoccidial shuttle programs are employed. The pattern of lesion development also demonstrates the danger of using anticoccidial shuttles too quickly within a flock, resulting in incomplete immunity and the reappearance of coccidiosis lesions before slaughter. Weight and feed conversion performance of these broilers (pure vaccination and vaccine-shuttle) compared favorably to conventional anticoccidial programs demonstrating the efficacy of these coccidiosis control strategies. Of all US broilers placed in 2014, 13% used coccidiosis vaccine. The number increased to 25% of all broilers placed in 2015, attesting to the economic performance success of coccidiosis vaccination programs.

Keywords: coccidiosis, vaccine, vaccine- anticoccidial shuttle

S3- 0081 Isolation and molecular characterization of a virulent serotype I Marek's disease virus from Andhra Pradesh, India

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Marek's disease (MD) is one of the most significant neoplastic diseases of poultry caused by Marek's disease virus (MDV), an oncogenic avian herpes virus which is responsible for great economic losses to the poultry industry worldwide. The disease was earlier described as paralytic disease characterized by flaccid paralysis of limbs with gross enlargement of peripheral nerves. But in the recent outbreaks, MD is being manifested as an acute disease with lymphomas in multiple visceral organs. In the present study, an outbreak of MD was investigated in one of the poultry farms from Andhra Pradesh, India. The gross lesions in the affected birds included lymphomas in different visceral organs like liver, spleen, proventriculus, heart and ovaries. Histopathological studies revealed presence of uniform lymphoblastoid cell infiltration typical of Marek's disease. The buffy coat was separated from the blood samples of affected birds and the isolation of the virus was carried out in duck embryo fibroblast cells. After three blind passages, the cell cultures revealed plaque formation typical of MDV. Further confirmation of the virus was carried out by PCR targeting 132 bp repeats of serotype-1 MDV and the oncogenes Meq and vIL-8 were amplified and sequenced. The nucleotide and phylogenetic analysis of the virus confirmed the virus as virulent serotype - 1 MDV. The present outbreak suggests the need for serotype-1 MD vaccination in Indian poultry flocks as the HVT and bivalent vaccines are unable to protect the flocks against virulent MDV.

Keywords: isolation, characterization, MDV, India

S3- 0082 Virus neutralization study using H120, H52, 793/B antisera on Iranian infectious bronchitis virus genotypes

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Avian infectious bronchitis (IB) is an acute and highly contagious respiratory disease of chickens. The disease is caused by avian infectious bronchitis virus (IBV), a coronavirus, which mainly affects the respiratory tract of chickens. IBV varies greatly genetically and phenotypically while the number of IB serotypes appears to have increased in recent years. Molecular studies have shown that a new IB serotype can emerge as a result of only a very few amino acid changes in the S1 part of the spike genome of the virus. Infectious bronchitis is currently one of the most important diseases in the poultry industry in Iran. The aim of this study was to investigate the neutralizing efficiency of H52, H120 and 4/91 vaccines against IBV variants circulating in Iran. First, samples were collected from IB suspected flocks and their genotypes were determined. Then antisera were prepared against different serotypes of IBV in SPF chickens. The virus neutralization test was conducted and the neutralization index was determined based on Kerber method. As the result, H52 and H120 antisera failed to neutralize variant 2 whereas 4/91 was partly neutralized. Also, variant 2 specific antiserum could neutralize the variant 2 isolate. Meanwhile, H52 and H120 specific antisera did not neutralize the IR-1, but 4/91 antiserum neutralized IR-1 whereas IS720 specific antisera neutralized IS720 genotype. However, H120, H52 and 4/91 antisera did not. Finally, it is necessary to mention that antisera against 793 / B isolate did not neutralize H120 but 4/91 and 793 / B antisera were able to neutralize it with the NI indexes of 3.5 and 5.5. According to the results of our study, development of effective vaccines using novel strains is recommended.

Keywords: avian infectious bronchitis, virus Neutralization, variant 2

S3-0083 Antibiotic resistance of *Salmonella* spp isolates from Iran's broiler farms

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Salmonella is the most important pathogen that involves in human's food borne diseases with 3 possible consequences as enteric fever, gastritis and extra enteric infection. For appropriate treatment of sick poultry, it is necessary to determine the antibiotic resistance of bacteria. To do so, disk diffusion method as one of the most common antibiogram methods was used to consider antibiotic resistance of salmonella isolates from broiler chicks in Iran. A total of 897 chicks from 1 to 45 day-old birds of 378 broiler farms with clinical signs and necropsy finding of salmonellosis were collected during 2013-15 and then were transported to laboratory. After that, the samples were cultured according to standard operation method. Antibiotic resistance to a panel of antibiotics was determined through disk diffusion method for salmonella isolates. Due to bacterial resistance to antibiotics, diameter of lack growth halo varies. Results of antibiogram test were reported as sensitive, moderate sensitive, intermediate and resistant classes. The study results showed that the most sensitive antibiotic was Fosfomycin, followed by Enrofloxacin and Doxycycline. The relationship between the occurrence of resistance and the consumption of the antimicrobial was studied. The results showed the present level of resistance to growth promoters in bacteria from food animals in Iran. This study reports higher rate of antibiotic resistance *Salmonella* spp isolates from day old broiler chicks and demonstrates the continuing evolution of resistance to antimicrobial agents.

Keywords: antibiotic resistance, *Salmonella* spp, broiler

S3-0084 Recirculation of pancreatic enzymes in broiler chicks

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A trial was conducted on Cobb 500 broiler chicks. All birds have received a standard ration in accordance with Cobb 500 broiler management guide. 20 chicks were operated after 20 days of age to sample the pancreatic juice (method of Ts. Zh. Batoev and S. Ts. Batoeva, 1970). Duodenum was fistulated to sample duodenal chymus. Physiological study of secreting activity of pancreas and intestinal digestion was performed during 180 min. Blood from axillary vein was sampled after 30 min., bile was sampled after slaughter. The activities of amylase (Smith, Roy, Ugolev, 1965), protease (Ts. Zh. Batoev, 1970), and lipase (biochemical analyzer Chen well 2900 (T), "Human" reagent kit (Germany)). The statistical analysis of the results was standard with the use of Student's t-test. Experimental data showed that activity of digestive enzymes in broiler chicks is maximal in pancreatic juice. In duodenal chymus amylase activity is 22.9%, protease 18.6%, lipase 123.4% compared to the respective activities in pancreatic juice. The results of our study showed that only a minor part of activated enzymes (1.3-10.0%) is excreting with feces while the rest is recirculating into the blood stream and releasing into the duodenum with pancreatic juice and bile. The closest amylase/protease ratio in pancreatic juice and blood serum was determined; this parameter could be used in diagnostics of physiological condition of the pancreas in chicken.

Keywords: pancreatic enzymes, pancreatic juice, bile, blood serum, broiler chicks

S3-0085 Effects of different dietary levels of propolis on performance, carcass characteristics and immunity response of broiler chickens

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Six hundred one-day-old broiler chicks were randomly assigned to six groups. Each group was five replicates with 20 birds per replicate. The dietary groups consisted of the supplementation of the basal diet with 0, 0.5, 1.0, 1.5, 2.0 and 3.0 g/kg diet of propolis. At 42 d., 10 chickens per each group were selected and slaughtered to determine the carcass characteristics. On d. 21 and 42 two chicks of each replicate were randomly selected and their blood samples were collected. The separated serums by centrifugation were used for antibody titration against Newcastle viruses. The results indicated that birds diet supplementation with propolis increased body weight and feed intake ($P<0.05$) and also improved feed efficiency ($P<0.05$) during the experiment. Mortality was significantly greater in the control group than in the experimental groups. The carcass yield values showed significant effects of dietary propolis on the eviscerated carcass percentage. There is significant difference ($P<0.05$) between antibody content of the serum against Newcastle disease (ND).

Keywords: propolis, broiler, immunity response, performance

S3-0086 Berberine ameliorates cartilage degeneration in dithiocarbamate-induced tibial dyschondroplasia via angiogenic pathway

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Tibial dyschondroplasia (TD) is an important long bone disorder of fast growing birds that disturbs the proximal growth plate characterized by non-vascularized cartilage, distended growth plate and lameness. Berberine is an isoquinoline alkaloid of the protoberberine type being used as an antifungal, anti-inflammatory and antitumor agent. Its therapeutic effects on bone healing have recently been discussed in various studies. The present study was conducted to investigate the ameliorative effects of this herbal medicine on TD-affected chicken broilers. The chicks were divided into two groups; (A) control group ($n=100$) with normal diet, (B) thiram group ($n=150$) which received a normal diet with 40 mg/kg of tetramethyl thiuram disulphide (thiram) to induce TD. On day 7 post-hatch, half of birds from the thiram group were separated and designated as (C) berberine group and administered with berberine (25 mg/kg/day) orally. Slaughtering was done on day 7 and 14. The angiogenesis in affected tibial bone was evaluated by the expressions of hsp90, VEGF and (VEGF receptor) Flk-1 by q-PCR and protein levels of hsp90 were measured by western blot analysis. The cartilage degeneration and amelioration was assessed by measuring the levels of CD147 and matrix metalloproteinase-9 (MMP-9). The expression levels of hsp90 and VEGF mRNA transcripts were increased while Flk-1 receptor, CD147 and MMP-9 were decreased in TD-affected chicks. However, the berberine therapy inhibited the hsp90 mRNA and protein levels and up-regulated the expressions of receptor Flk-1, CD147 and MMP-9 in TD-affected tibial growth plates significantly ($P\leq 0.05$) resulting into the angiogenesis and normal bone growth. In conclusion, our study suggests that the administration of berberine to dyschondroplastic chicks has resulted in preventing the un-vascularized growth plate and lameness; and ultimately reinstated the angiogenesis making this herbal medicine an efficacious drug for the treatment of TD.

Keywords: tibial dyschondroplasia, berberine, angiogenesis, chicken broilers

S3-0087 The measurement of chicken acute phase proteins serum amyloid-A, C-reactive protein, ovotransferrin, transthyretin, haemopexin and apolipoprotein A-1 using a quantitative proteomic approach

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This study aimed to establish a quantitative proteomic method to measure acute phase proteins (APPs) serum amyloid A (SAA), C-reactive protein (CRP), ovotransferrin (OVT), transthyretin (TTN), haemopexin (HX), PIT54, apolipoprotein A-1 (Apo-A1) and apolipoprotein A-4 (Apo-A4) in chickens. The measurement of APPs in chickens is challenging owing to the limited availability of specific antisera and low abundance in serum. Three acute phase groups: highly acute phase (HAP), acute phase (AP) and non-acute phase (NAP) underwent protein fractionation and phospho-lyl choline enrichment to purify CRP. Enriched fractions and pooled samples of HAP, AP and NAP serum were trypsin digested and applied over 2D to a Q-Exactive Hybrid Quadrupole - Orbitrap mass spectrometer (MS) (Thermo Scientific) in an untargeted screen. A Q-Exactive-Orbitrap MS performed MS2 spectra analysis and comparisons were made to the Gallus gallus protein database (Uniprot) using Mascot (Matrix Science). Quantotypic peptides for individual samples of HAP (n=3), AP (n=4) and NAP (n=4) were then measured in a targeted screen to produce peak areas and Skyline v.3.5 (MacCoss) integrated the data and determined the relative intensities of each peptide. A Kruskal-Wallis test (Prism v.5) found all the SAA peptides and three OVT's peptides were significantly different between the groups, confirming SAA and OVT as positive APPs. Apo-A1 behaved as a negative APP, with significantly lower abundance in the HAP samples for all three peptides confirming for the first time that this is a negative APP in chickens. TTN behaved as a negative APP and PIT54, HX and Apo-A4 showed no significant differences between the acute phase groups. C-reactive protein was not identified suggesting it is not an APP in chickens. This study identified quantotypic peptides for seven APPs enabling this method to be readily used in future studies, allowing a large number of APPs to be measured without the limitations of immunoassays.

Keywords: acute phase proteins, proteomics, quantitative

S3-0088 The effect of microbial challenge on the intestinal proteome of broiler chickens

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The aim of the study was to characterise the chicken small intestinal proteome over time as a result of a mixed microbial challenge using a 2-D DIGE method. 120 day old chicks were divided between 12 pens, on day 12 re-used litter, shown to contain *Campylobacter* and coccidial oocysts to affect an enteric challenge, was added to 6 pens. On days 12, 15, 18 and 22 each pen was weighed and the jejunum of one chick recovered and pooled to create a challenged sample and a control sample at each of the four time points. Protein extracts from the samples were bound to CyDyes 2, 3 and 5 and ran on duplicate gels. DeCyder v7.0 BVA used two-way ANOVA determined which spots were differentially expressed between challenge and control samples over time. As well as significantly affecting growth, the addition of the re-used litter caused differential expression of a large number of intestinal proteins. Villin-1, an actin associated protein involved in apoptosis was reduced in expression in the challenged birds indicating that many of the changes in cytoskeletal protein expression in the challenged birds were as a result of an increased rate of apoptosis. Heat shock proteins decreased in expression over time in the intestine and this was more pronounced in the challenged birds. It is unclear what biological processes occurred to cause this reduced expression, as similar studies have found stress induced proteins such as heat shock proteins increase in expression. The small intestinal proteome sampled from 12 to 22 days of age showed a great deal of developmental change. The results are comparable to similar studies in other species and indicate that many of the responses to infection and inflammation in the small intestine are conserved among vertebrates. Detailing changes in protein expression give further insight into how changes in intestinal health effect the growth of the bird as well as identifying potential biomarker targets.

Keywords: proteomics, gut health, broiler

S3-0089 Reviews of poultry respiratory health in Asia

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The respiratory problem presents a continuing, potential threat for economic loss in most of the poultry industry in Asia. Factors including proper management, the control of infectious pathogens such as Newcastle Disease (ND), both highly pathogenic and low pathogenic Avian Influenza (AI), Infectious Bronchitis (IB), Infectious laryngotracheitis (ILT), Avian metapneumovirus (AmpV) infection, Infectious Coryza (IC), and Mycoplasmosis (MS, MG) were reviewed. Interaction amongst pathogens such as MS and IB was highlighted. Other factors immunosuppression (Chicken anemia, Gumboro Disease and Mycotoxins), intestinal health (Coccidiosis and Necrotic Enteritis) should also be reviewed for a better control of respiratory problem. The challenge of genotype VII ND and its control with classical ND versus homologous vaccines; the protectotype concept in the face of IB variants challenge, the impact of low pathogenic AI concurrent infected with other pathogens such as IBV, NDV were reviewed. Besides the infections, management especially the ventilation is also playing a major role in the respiratory health. The ventilation is getting more problems typically in extreme climate conditions. Additionally, a proper differential diagnosis should also be implemented to ensure the correlated measurement for the correction of respiratory problem is appropriately correct. The data both in laboratory results and performance should be well analyzed after each flocks to have a better understanding of the message from the chicken, building up the farm level baselines is essential to ensure the respiratory health of the birds and the economic performance. The selection of vaccines and proper vaccination skills should be monitored for fine tuning the vaccination program in practice. In conclusion, poultry respiratory problem is a complicated issue and it needs a 360o approach to look for a better solution.

Keywords: respiratory problem, immunosuppression, intestinal health

S3- 0090 Oxidative damage caused by inorganic arsenic and its amelioration with vitamin E and bentonite in broiler chickens

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The present study was executed to investigate adverse effects of inorganic arsenic in broilers and to ascertain role of vitamin E and bentonite in alleviating its harmful effects. For this purpose, the experiment was conducted on 140 one-day-old broiler chickens. Birds of groups 2, 5, 6 and 7 were administered inorganic arsenic @ (50 mg. kg-1 BW) through feed. Groups 3, 5 and 7 received vitamin E (150 mg.kg-1 BW) and groups 4, 6 and 7 received bentonite (5 mg.kg-1 BW), respectively. Group 1 was kept as control. All the birds treated with inorganic arsenic (group 2) showed a significant decrease in haematological parameters at day 21 and 42. Biochemical parameters i.e. total protein, albumin and globulin were decreased while ALT, AST, urea and creatinine were increased at day 21 and 42. Total antioxidant capacity and Catalase were decreased, while MDA and Total oxidant status levels were increased in inorganic arsenic treated group (group 2) at day 21 and 42. Co-administration of bentonite along with inorganic arsenic resulted in partial amelioration (group 6) as compared to group 5 and 7 administered inorganic arsenic+vitamin E and inorganic arsenic+vitamin E+bentonite, respectively. It was concluded that inorganic arsenic causes damage not only to haematobiochemical parameters but also to oxidative parameters in broilers and it can be ameliorated with vitamin E administration while partially amelioration occurs with bentonite. Combination of vitamin E and bentonite is the best in combating inorganic arsenic toxic effects.

Keywords: broiler chickens, arsenic, oxidative damage, vitamin E, bentonite

S3-0091 Cleaning and disinfection in broiler house: efficiency and productivity against *Campylobacter jejuni*

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The aim of this study was to evaluate the productivity and the efficiency of cleaning and disinfection program for broilers challenged with *Campylobacter jejuni*. Two subsequent lots were made, with 960 birds each, divided into 32 boxes with 30 birds each. In the first lot, all the birds were challenged inoculated orally with 105 cfu/ml *Campylobacter jejuni* strain atcc 33560. In the second housing, before housing chicks, two cleaning and disinfection programs were performed: Proposed (washing with acid and alkaline detergent at 4%, application of glutaraldehyde disinfectant 250g/L + formaldehyde 185g/L at 0.5% and disinfectant paraclorometacresol 210 g/L) and Common (wash with mild detergent to 4%) in 16 boxes each program. In the second lot, total microorganism count was done in facilities before and after the cleaning and disinfection. At slaughter was evaluated the frequency of occurrence of *Campylobacter* spp. in living birds and in the carcasses after plucking and chiller. Broiler performance was also evaluated. After carrying out the procedures, a lower total microorganisms count was found in the Proposed program in feeders (0.67 vs 3.42 log₁₀/CFU cm², P=0.0044), walls (0.975 vs 3.099 log₁₀/CFU cm², P=0.0076) and floor (0.143 vs. 3.865 log₁₀/CFU cm², P<0.0001). There was no difference in the frequency of occurrence of *Campylobacter* spp. Between the treatments for live birds (7.5% vs. 20%, P=0.07) and carcass after plucking and chiller (20% vs. 5%, P=0.121 and 10% vs. 10%, P=1.00). Birds housed under the Proposed conditions had higher body weight (2,717 vs. 2,532g, P=<0.0001), weight gain (2,610 vs. 2,447g, P=0.002), feed intake (4,903 vs 4,760g, P=0.035) and better feed conversion (1.88 vs 1.95, P=0.05) in the period of 1-42 d. Thus, the Proposed protocol for cleaning and disinfecting provides greater reduction of microbial load of broiler facilities and positively influences the productive performance of broilers challenged with *Campylobacter jejuni*.

Keywords: biosecurity, campylobacteriosis, disinfectants, facilities, health

S3- 0092 Reviews of poultry intestinal health in Asia

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Reviews are made on the factors that should be considered in the maintenance of intestinal health. Although measures including in-feed ionophore and chemical anticoccidials have been widely applied to control coccidiosis, it is still a major problem in the field due to its reduction in sensitivity. Thus, a proper control of coccidiosis with the use of coccidiosis vaccine is highlighted; the key factors for a successful coccidiosis vaccination such as the application method of the coccidiosis vaccine with the specific sprayer in the hatchery, litter management, stocking density and a consistent monitoring with OPG and lesion scoring are also introduced. Amprolium routinely used within 14-15 days after the vaccination could disturb the cycling of the oocysts, could affect the building of immunity thus a vaccine failure is commonly seen. Necrotic enteritis (NE) induced by *Clostridium perfringens* is found mainly either after a break of coccidiosis or an unsuccessful use of coccidiosis vaccine. Most of the in-feed antimicrobial growth promoters (AGPs) could help to minimize the severity of NE, however, attention should be paid on the building up of drug resistance, while Enradin (enramycin) is still proven to have consistent minimum inhibition concentration (MIC) results on *C. perfringens* since 1986. Other viral pathogens that could induce mal-absorption or runting-stunting syndrome are also getting more attention in the field. The controls immunosuppression factors should also be thoroughly reviewed. In conclusion, the intestinal health starts with a good analysis of all the possible enteritis causing factors, relevant plans of control measurements, execution of the control plan and followed and fine-tuned by a good and consistent monitoring. The control measures include production of good quality feed with good quality raw materials, management, a good control and monitoring program of coccidiosis and the use Enradin in the feed for the prevention of NE.

Keywords: coccidiosis, intestinal health, necrotic enteritis, coccidiosis vaccine

S3-0093 A wax matrix is an efficient butyrate carrier that can be used as a feed additive to reduce Salmonella colonization

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Butyrate has been used extensively as a feed additive to decrease Salmonella colonization in poultry with inconsistent results. Discrepancies in the literature could be due to butyrate release location effects. In this study, newly developed butyrate formulations were evaluated for their effect on shedding and colonization of broiler's digestive and immune tissues by Salmonella Enteritidis, and for butyrate release in the gastrointestinal tract. Broilers were randomly allocated to 7 dietary treatment groups: 1) control without butyrate, 2) novel wax matrix, 3) novel wax and starch matrix, 4) polyhydroxybutyrate (PHB), 5) pH-sensitive polymer coated butyrate (SPH), 6) tributyrin and 7) commercially available fat-coated product. The formulations were based on different approaches to modify butyrate release profile. Release from wax matrices is based on diffusion/erosion and is nearly pH independent, inducing a sustained release. PHB is hypothesized to be enzymatically degradable to release 3-hydroxy butyric acid throughout the GIT. The SPH formulation supposedly release butyrate around pH 7 in the colon. Tributyrin is based on the hydrolysis of esters to release butyric acid targeting small intestine. The fat-coated butyrate was included as a comparator, because of its known effect on reduction of Salmonella colonization. Each chicken was orally inoculated with 105 cfu/mL of S. Enteritidis at day 17 post-hatch. Four days after infection the number of cfu Salmonella per g cecal content and spleen were determined. Wax matrix without starch reduced the Salmonella colonization in cecal content with > 1 log. A trend in reduction of cfu/g S. Enteritidis was observed for the fat-coated butyrate in cecal content (0.9 log). Increases in butyrate concentration measured in the caeca correlated with reductions in Salmonella colonization. In conclusion, new formulations of butyrate that increase its release in the ceca seem promising to protect against S. Enteritidis colonization.

Keywords: broilers, Salmonella, butyrate

S3- 0094 Serological prevalence in diseased flock exhibiting immunosuppression and respiratory tract infection cases in India

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Poultry is one of the fastest growing segments of the agricultural sector in India but in the past two to three years, the industry is facing losses due to immunosuppressive diseases along with respiratory infections and severe drops in egg production. On the basis of clinical signs and post mortem lesions from immunosuppressive flocks Chicken Anemia Virus (CAV) ; from swollen head with respiratory complications and egg drop cases Avian Pneumo Virus (APV) ; infection with respiratory sound, gasping with egg drop cases Infectious Laryngotracheitis (ILT) were suspected and subjected to serological diagnosis. Since no vaccines are available to control the above infections in India, the serological data (ELISA) predicts the prevalence of the challenge viruses for CAV, APV and ILT circulating in India Poultry disease cases. Serum samples from suspected clinical cases were collected from Breeder, Broiler and Layer farms from different parts of the country during 2012 to 2015. CAV is a vertically transmissible disease and from 60 farms selected for sero-prevalence a total of 62% flocks were confirmed positive. In respiratory infection cases, 277 farms were suspected for APV and out of those farms, 76% flocks were confirmed positive. In a total of 265 farms suspected for ILT, samples showed 94% of sero-prevalence. Above study indicates the need of good quality vaccines to control CAV, APV and ILT infection.

Keywords: immunosuppression, seroprevalence, vaccination

S3- 0095 Cross- species infection of avian leukosis virus subgroup J in pheasants and quails

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To demonstrate the potential cross-species transmission ability of avian leukosis virus subgroup J (ALV-J) under host pressure, we used five avian species, SPF chicken, black turkey, seven-color pheasant, Japanese white quail and Cherry Valley duck as host pressure model for ALV-J cross-species infection. The first two species are susceptible, and the latter three species are resistant to ALV-J. Embryonated egg of SPF chickens were inoculated with the stock virus of ALV-J (P0), and then hatched and monitored the infection status until the mutants were isolated. The isolated strain was named P1 and then inoculated into embryonated eggs of next species and conducted same experiment with previous species. After hatching, cloaca swabs and blood were taken at interval day from day 1 for group specific antigen (p27) test and virus isolation. All birds were killed at terminal day. The results showed that the resistant host of pheasant and quail were successfully infected through the stepwise continuous host pressure. However, ducks were not infected. The infection rate of SPF chickens and turkeys was both 100% (16/16), while those pheasant and quail was 37.5% (6/16) and 11.1% (3/27), respectively. Compared with the origin strain, the amino acid substitutions of all isolates were distributed throughout the envelope glycoprotein, and there was a clustering of sequence variations in hr2 domains of the gp85 gene. By nonsynonymous mutation and synonymous ratio (NS/S) analysis, the value of NS/S in hypervariable regions (hr2) of isolates from pheasant and quail was 2.5, respectively. It indicated that the mutation of isolates in pheasant and quail was induced by selective pressure from the resistant host, and the hr2 region was responsible for cross-species infection of ALV-J. It indicated that pheasant and quail can provide sufficient selective pressure for ALV-J mutation, so they are good model for further study the mechanism of cross-species transmission.

Keywords: avian leukosis virus subgroup J, cross-species infection, host pressure

S3- 0096 Discovery of pathogenic *Macroccoccus caseolyticus* in commercial broiler chickens

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A pathogenic *M. caseolyticus* was discovered in commercial broiler chickens. In September 2015, ten 38-day-old sick broiler chickens from five 15000-chicken flocks were sent to our lab for diagnosis. Grossly, yellow caseous exudation was discovered under the skulls. Microscopically, meningoencephalitis and necrosis were observed in the cerebrum and cerebellum. Numerous eosinophilic granulocytes surrounded various vessels. Lymphocytic foci infiltration was present in the liver and proventriculus. A kind of bacterium was isolated on LB agar. The isolates showed Gram positive staining and globular morphology with size of 1.3-1.5 μ m. The bacterium was identified by PCR with 16S rRNA primer. The sequence of 16S rRNA was BLAST and the result showed that all the ten isolates are *M. caseolyticus*, and their sequence showed 100% identity. The strain was named SDLY. Alignment analysis of amino acid sequence of *mecB* gene in SDLY strain showed that it contained a cluster mutation (6 amino acids) between No.170 and No.200 amino acid (accession number: KU573955), indicating the responsibility for the pathogenicity of SDLY strain. The agardisc diffusion susceptibility test for antibiotic resistance pattern of SDLY showed twenty six resistant antibiotics and only four sensitive antibiotics, indicating it possible is a superbacterium. Experimental inoculation of the SDLY strain into SPF chickens showed similar lesions with field cases. This is first report of pathogenic *M. caseolyticus* in commercial broiler chickens with inflammation and necrosis. Whether the strain of *M. caseolyticus* represents a prevalent virulence factor or an opportunistic pathogenic pathogen in chickens, it still remains to be further investigated. Nevertheless, a cluster mutation in *mecB* gene of *M. caseolyticus*, and the massive presence of *M. caseolyticus* in broiler chickens indicated that more attention should be paid to this microorganism in poultry and public health.

Keywords: *M. caseolyticus*, commercial broiler, *mecB* gene, mutation

S3-0097 Case report – Leucocytozoonosis infection in layer hens

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5 layer hens were received from Huimin County for disease diagnosis by the Shandong Agricultural University Animal Molecular Pathology Laboratory. Clinical signs were low production, daily gain and feed intake decrease. All birds had been vaccinated for Newcastle disease vaccine and had received a dose each for Avian influenza vaccine. For histopathology, tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 mm, mounted on glass slides, and stained with hematoxylin and eosin (H&E) by routine methods. For molecular pathology, total RNA was abstracted and some suspected virus was detected by reverse transcription polymerase chain reaction (RT-PCR). Results: Pathological changes are mainly liver swelling, punctate hemorrhages, partial color khaki, dim texture toughness, fragile; the proventriculus papilla shows edema and adhesion with obscured structure; There were many punctate hemorrhages in the spleen. Histopathological examination, Severe fatty degeneration and necrosis were presented in liver cells, bile duct epithelium hyperplasia and infiltration of inflammatory cells to different extent; We found homogenization and necrosis of smooth muscle of duodenum and inflammatory cell infiltration between myocardial fibers. The blood smear examination showing gametocytes. According to the pathological features, this case suspected belongs to the ALV-J and Leucocytozoonosis at first. Molecular diagnosis RT-PCR identification of ALV-J and The ALV-J negative. There was a case of suspected Leucocytozoonosis infection. The vectors are Simulium or Culicoides species. The current prevention method is vector control and clean up the environment. SQ, SMM, SMD-TMP can produce good curative effect.

Keywords: leucocytozoon, leukocytosis, layer hens

S3-0099 Isolation and molecular identification of paramyxovirus-1 (PMV-1) from Ahvaz pigeons and evaluation pathogenicity of isolated virus in broiler chickens

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In order to isolate and identify pigeon Paramyxovirus-1 (PPMV-1) phylogenetically and evaluate the virulence of the isolated virus in broiler chickens, 232 samples from cloacal and oropharyngeal swabs of 22 live pigeon lofts suspected to Newcastle disease (ND) and from trachea, lung, liver, spleen, kidney, brain, proventriculus and cecal tonsil of died pigeons from 28 lofts were collected in Ahvaz city - Iran. Virus isolation was done into the allantoic cavity of embryonated chicken eggs. RNA extraction and cDNA synthesis were conducted. With PCR, multiplication of cleavage site of F gene was carried out and PCR products were sequenced and phylogenetic comparison on isolates was performed. In order to study pathobiology of isolated PPMV-1, one hundred sixty one- day- old broiler chicks were divided into 4 equal groups. Group 1&2 chicks vaccinated against ND by B1 vaccine at 9 days. Groups 3&4 were kept as unvaccinated control groups. Group 1&4 chicks were challenged with 105EID₅₀ of highest virulent isolated PPMV-1 by ocular route at 29 days. The results showed PPMV-1 is enzootic in Ahvaz pigeons and analysis of F0 cleavage site on 12 isolates indicated that all isolates are virulent NDV with 112KRQKRF117 motif. According to chicken embryos mean death time, all isolates were mesogenic and chickens challenged with most virulent isolate, showed respiratory signs, conjunctivitis and in some cases depression and lethargy and induced humoral immune response in chickens. It was concluded that based on molecular characterization, isolated PPMV-1 is virulent NDV and caused mild ND in chickens and B1 vaccine protected chickens against PPMV-1 infections.

Keywords: isolation, broiler chicken, Newcastle disease, pigeon paramyxovirus-1

S3-0101 Isolation and identification of a highly pathogenic Muscovy duck parvovirus

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A Waterfowl Parvovirus(MPV-A) isolated from muscovy duck with symptoms of high mortality rate ,yellow-green or white diarrhea and respiratory symptoms on Muscovy Duck farms in Anhui by duck embryo inoculation belongs to highly pathogenic Muscovy Duck Parvoviruse. Pathological changes with spleen necrosis, liver enlargement, kidney and pancreas hemorrhagic necrosis were observed. 10-day-old Muscovy ducklings was inoculated respectively with three dilutions(103EID50,104 EID50,105 EID50) of purified virus which appeared the same clinical symptoms and pathological changes compared with field cases, and MPV-A was recovered from the tissues of infected ducks. In addition to, Animal experiments showed a high mortality rate that each group at least 80%. Polymerase chain reaction (PCR) were applied with whole genome amplification, sequencing results showed 96.5%-99.6% and 95.4%- 98.7% homologies between MDPV isolates at nucleotide and amino acid level, respectively. In VP protein, MPV- A have 3 glycosylation sites, and there are missing more amino acid deletions sites, presumably related to high virulence.

Keywords: high virulence, mdpv, isolation and identification

S3-0102 Issue of antibiotic resistance in commercial poultry of least developed countries : A case from Nepal

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The continuous use of antibiotics in compound feed has been an integral part of commercial poultry production in several least developed countries including Nepal which promotes bacterial resistance towards antimicrobial agents. Hence, this study was designed to determine antibiotic resistance in commercial poultry of Nepal taking *E. coli* as flagship bacterium. The live/dead birds carried to veterinary teaching hospital of Agriculture and Forestry University in Nepal by commercial poultry producers were our cases. We conducted clinical examination of live/dead birds followed by postmortem examination and necessary laboratory investigation(s). The birds diagnosed with colibacillosis on presumptive diagnosis were included in sampling frame. Each sample represented different commercial farm and in total sample size was 80 (40 broiler, 40 layer). The liver from each sample were isolated for culture, identification and confirmation of avian pathogenic *E. coli* followed by evaluation of antibiogram using standard protocol. The data collection and analysis was conducted using SPSS 16.0 version. In this study, we found that *E. coli* has developed resistance most substantially towards Cephalixin and Amoxycillin (n=65, 81.2%) followed by Tetracycline (n=63, 78.8%), Colistin sulphate (n=50, 62.5%). Chloramphenicol (n=49, 61.2%), Ciprofloxacin (n=44, 55.0%), Enrofloxacin (n=43, 53.8%), Levofloxacin (n= 23, 28.8%), however, they have not developed resistance against Amikacin. The proportion of *E. coli* isolates that have developed resistance against Colistin sulphate ($p<0.05$), Chloramphenicol ($p< 0.05$), Tetracycline ($p<0.001$), Ciprofloxacin ($p<0.01$), Enrofloxacin ($p<0.05$) and Gentamicin ($p<0.01$) was significantly higher in layers compared to that of broilers. In conclusion, this antibiotic resistance in avian *E. coli* against several antibiotics indicates serious food safety issue, resistance transmissibility and treatment failure in poultry, livestock and human.

Keywords: antibiotic, resistance, poultry, nepal, *E. coli*

S3- 0104 Experimental study on CBC and serological test in ostriches infected by IBD and AI subtype H9N2

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This study discusses avian influenza (AI) and Infectious Bursal Disease (IBD) in ostriches and its influence on CBC and antibody titers. The HI and ELISA testes were checked and comprised with CBC in challenge with Infectious Bursal Disease (IBD) and Avian Influenza (AI) sub type H9N2 viruses in ostrich. 8 ostrich chicks about 1 month were prepared. After 10 days adaptation, they were divided into 2 pen/4 birds. Group 1 received IBD virus and group 2 received AI (H9N2) virus. Then at 2 days prior to challenge and 7 and 18 days post-challenge sera samples and blood smears were prepared from group 2 and at 2 days prior to challenge and 3, 11 and 21 days post- challenge sera samples and blood smears were prepared from group 1. The results were evaluated and analyzed by Sigma Stat software. In the group 2, HI test significantly increased at 7 day post-challenge compared with 2 day prior to challenge but CBC didn't show a significant change in before and after the challenges. In the group 1, ELISA test significantly increased at 21 day post-challenge compared with 2 day prior to challenge and 11 day post-challenge but CBC didn't show a significant change in after and before the challenges. The present study show that infectious bursal disease infected the ostrich but didn't any effects on CBC. HI and ELISA testes respectively are useful technique for detection of IBD and AI antibody after challenges in ostrich.

Keywords: ostrich, CBC, AI, IBD

S3- 0105 Epidemiological investigation of avian leukemia to some indigenous chicken

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To learn Avian Leucosis virus infection status of Chinese indigenous chicken for providing the basis to purifying of the disease, the ELISA kits and virus isolation method were applied to detect the levels of ALV- AB and ALV- J, which belong to the major exogenous avian leukemia virus. 3557 samples were collected from 10 different indigenous chicken of China. To further tracking infection status of ALV, the offsprings of 4 indigenous chicken were also detected. At the same time, the used vaccine in the feeding process was also detected if polluted by ALV. The results showed the positive rate of ALV- P27 antigen ranged from 0% to 62.1%, the ALV- AB from 0% to 25.0%, the ALV- J from 0% to 59.0% and the ALV isolation from 0% to 22.0%, and the positive rate of ALV- P27 antigen from the offspring was 6.0% to 67.0%, the positive rate of ALV isolation from 2.0% to 34.3%. The results also showed 2 kinds of vaccines from 2 batches produced by a manufacturer were polluted by ALV. The study indicated that the indigenous chickens can be infected by ALV in large group, there is great difference for the positive rate of ALV among different chicken breeds which may be related to inheritance of resistance. The ALV positive rate of some offspring chicken even slightly improved perhaps related to the polluted vaccine.

Keywords: indigenous chicken, avian leucosis virus, epidemiological investigation

S3- 0106 Enradin keeps lower MIC advantage– consistent MIC results of Enradin against *C. perfringens*

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Despite the great advances achieved in the last few decades in many different areas such as management, health and nutrition, poultry industry remains challenged by clostridial infections, present in a great percentage of broiler flocks. Left uncontrolled, bacteria from genus *Clostridium* – especially *C. perfringens* type C - can lead to necrotic enteritis (NE), cholangiohepatitis and gangrenous dermatitis, conditions that could have a devastating effect on broiler performance and processing yield. Gram-positive antibiotics have been used to manage those challenges in poultry for many years. With the rising concerns about bacterial resistance and its consequence for both human and animal health, keep monitoring for resistance profile of *Clostridium* field samples is an important tool to ensure the efficacy and safety of gram-positive antibiotics currently in use in poultry production. Since 1986, MSD Animal Health and Kyodoken Institute Japan conduct a long term study, with the objective to evaluate minimal inhibitory concentration (MIC) evolution of selected gram-positive antibiotics on more than 850 *Clostridium perfringens* field strains. Samples are collected during summertime in different regions, representative of main broiler production areas in Japan. MIC₉₀ is estimated upon serial dilution for each one of different molecules. In 2015, Enradin®, brand of enramycin, presented lower MIC₉₀ in feces isolates (0.10 µg/mL) in comparison with zinc bacitracin (>100µg/mL), virginiamycin (0.78µg/mL) and avilamycin (>100 µg/mL). When compared with results from previous years, MIC₉₀ for Enradin® shown no significant change against strains collected in 2015, thus confirming no bacterial resistance was developed on samples analyzed. This profile makes Enradin® a safe, sustainable option to fight against *C. perfringens* challenges in poultry.

Keywords: broilers, clostridium, bacterial resistance, enramycin, minimal inhibitory concentration

S3-0107 Diagnosis and identification of a case of hybrid of Marek's disease

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Marek's S disease(MD) is a lymphoproliferative and demyelinating disorder of chickens caused by Marek's S disease virus(MDV). Clinical signs of MD showed chronic polyneuritis, immunosuppression, lymphomas in visceral organs and other tissues, transient paralysis and acute brain oedema. Its contains eye type, nervous type, viscerotoxemia type and skin type. Neoplastic diseases occurred in some farm in jilin province in September 2015. Marek's disease or Avian leukemia was preliminary diagnosed by epidemiological investigation, clinical symptoms and pathological changes. Furthermore, The liver and spleen of sick chickens was collected, and the genomic DNA was then extracted for polymerase chain reaction analysis by specific primers (MDV-F: 5-ATGATGCGATGCGATGAAAGTGCTATGGAG- 3,MDV- R: 5- ATCCCTATGAGAAAGC-GCTTGA-3;ALV-F: 5-G G AT GGT GGC TG ACTGTGT-3, ALV-R: 5CGAACCAAAGGTAA-CACACG- 3) and sequenced. Amplification bands is about 314 bp and the results demonstrated that the MDV is positive but ALV is negative. Accordingly, the disease was ultimately diagnosed with MDV infection. In addition to the infection is mixture of four type and mixture of typical pathological changes of each type. Rapid diagnosis of Marek's S disease executed by epidemiological investigation, clinical symptoms, pathological changes and laboratory tests. This study provides a basis for detection and control and of Marek's S disease.

Keywords: Marek's disease, hybrid, diagnosis, identification

S3-0108 Reduction of economic losses caused by colibacillosis in broiler chickens in Mauritius through vaccination

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This study describes the results of vaccination trials using a modified-live vaccine against *E.coli* administered as a coarse spray to broiler chicks between 1 and 3 days of age. The trials were carried out in one industrial broiler operation and two commercial/non industrial broiler farms. In the first trial, approximately 1 million broilers were vaccinated during 10 cycles of growth over a 20 months period. The broilers were raised under similar housing, feed and management conditions. Performance in terms of mortality, weight gains and feed conversion rates (FCR) was compared to the performance of unvaccinated birds kept in the same premises during 10 previous cycles. In the second trial, the performance of 14,000 vaccinated broilers was compared to the performance of 16,000 non-vaccinated birds kept in the same houses previously. In the third trial, 23,000 birds were not vaccinated and the performance compared with 7,500 vaccinated birds kept in a different farm but under the same management. On an average, vaccinated flocks showed a decrease of 3.73 % in mortality, an approximate increase of 0.27 Kg in live weight and an improved FCR of 0.327. The additional profit due to vaccination per chick housed in the industrial operation was 0.27 USD or a benefits/costs ratio of 13.3. The additional profits per chick housed in the other two farms were 0.52 USD and 0.77 USD respectively or benefits/costs ratios of 25.13 and 37.5 respectively. The study also estimates the economic losses due to colibacillosis at the national level based on excessive mortality, reduced performance, elevated food conversion rates, costs of treatment with antibiotics and excessive condemnations at the abattoir, at approximately 7.5 million USD annually.

Keywords: colibacillosis, economic losses, vaccination

S3-0109 The study of the activation of PI3K/Akt pathway by the σ A and σ NS protein of avian reovirus

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To find out whether ARV activate the phosphatidylinositol 3-Kinase-dependent Akt (PI3K/Akt) pathway according to the PXXP or YXXXXM motif of σ A and σ NS protein. Based on ARV protein amino acid sequence analysis, σ A protein have 4 PXXP (Amino acid 55-58, 110-114, 114-117, 200-203) and 2 YXXXXM (Amino acid 65-69, 207-211) motif, σ NS protein have 2 PXXP (Amino acid 159-162, 333-336) and 1 YXXXXM (Amino acid 179-183) motif. Gene splicing by overlap extension PCR was used to change the PXXP or YXXXXM motif of σ A and σ NS gene. Plasmid constructs that contain mutant σ A and σ NS genes were generated and transfected into Vero cells, and the expression levels of the corresponding genes were quantified according to immunofluorescence and Western blot analysis. The Akt phosphorylation profile of transfected cells were examined by flow cytometry and Western blot. The results showed that σ A and σ NS genes were expressed in the Vero cells, and the expression of P-Akt of the σ A mutant groups (Amino acid 110-114 and 114-117) decreased markedly. The results indicated that, the σ A protein of ARV activate the PI3K/Akt pathway by the PXXP motif (Amino acid 110-114 and 114-117). The results of this study reveal the mechanisms by which ARV manipulate the cellular signal transduction pathways, which may provide new ideas for novel drug targets.

Keywords: ARV, PI3K/Akt pathway, σ A protein, PXXP motif

S3- 0110 Genomic characterization of the newly emerged chicken parvovirus in China

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A previously unidentified strain of chicken parvovirus (ChPV) is now endemic among chickens in China. Analysis the genetic variation of the newly emerged ChPV by sequencing its full-length genome, to increase understanding of the origin and evolution of these emerging pathogens and provide a theoretical basis for the prevention and control of ChPV infection. According to the ChPV genomic sequences published in GenBank, 3 overlapping fragments to amplify the complete genomic sequence of ChPV by PCR from cloacal swab of the infected chicken and then cloned into vector pMD19- T and sequenced, respectively, the determined sequences were spliced and assembled by DNAMAN. Based on above efforts, the first complete genome sequence of GX-CH-PV-7 isolated from chicken was accomplished, and was further characterized by alignment with 10 published ChPV whole- genome sequences from GenBank. Moreover, recombination was analyzed by Simplot software. The complete genome of GX-CH-PV-7 (GenBank accession no. KU523900) is 4612 bp and composed of NS1, NP1, VP1 and VP2. GX-CH-PV-7 genome showed 89.0% – 93.9% nucleotide identity with 10 representative ChPV isolates. Phylogenetic analysis of the complete ChPV genome revealed 3 major clusters, the GX-CH-PV-7 strain was segregated into a distinct branch separate from other ChPVs and appeared to have a close relationship with Gallus gallus enteric parvovirus isolate ChPV 798 strain, from the United States, and formed a second cluster. No evidence for recombination between GX-CH-PV-7 and the other 10 ChPV strains, demonstrating that this strain was not generated through recombination from ChPVs. The first complete genomic sequence of the newly emerged ChPV was successfully characterized. The data will provide valuable theoretical reference for the prevention and control of ChPV infection.

Keywords: chicken parvovirus, genomic characterization, gene sequence analysis

S3-0111 Development of a novel immunochromatography test strip that uses Au/Fe₃O₄ core – shell nanoparticles for the rapid detection of avian influenza virus subtype H7

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Avian influenza is a highly pandemic disease amongst birds, small mammals and humans. In March 2013, the first case of human infection with avian influenza A H7N9 virus was reported by the Chinese Centre for Disease Control and Prevention. Since then, more than 450 human case of H7N9 infection have been reported. A novel immunochromatography test strip that uses magnetic particles constructed from Au/Fe₃O₄ core-shell nanoparticles was developed for the rapid detection of avian influenza virus subtype H7. The principle of this test strip is based on a sandwich immunoreaction in which AIV H7 antigens bind specifically to their corresponding antibodies on a nitrocellulose membrane. First, an antibody-Au/Fe₃O₄ core-shell nanoparticle conjugate that was used as a label was coated onto a glass fiber membrane, which was used as a conjugate pad. Next, to generate a test zone and a control zone, an anti-H7 polyclonal antibody and an anti-IgG antibody, respectively, were immobilized on the nitrocellulose membrane. Positive samples displayed red lines in the test and control zones of the nitrocellulose membrane, whereas negative samples resulted in a red line only in the control zone. The limit of detection (LOD) was 103.5 EID₅₀ after examination by the naked eye within 15 min. It was found that this test strip provides a lower LOD compared with an immunochromatography test strip that uses an antibody-gold nanoparticle as the label (LOD: 104.5 EID₅₀). In addition, 40 clinical samples were tested using our immunochromatography test strip to estimate its performance. The result showed that the accuracy and specificity of the test strip are both 100%. In sum, this test strip may offer a simple and cost-effective tool for the rapid detection of AIV H7.

Keywords: avian influenza virus subtype H7, Immunochromatography test strip, Au/Fe₃O₄ core-shell nanoparticle

S3-0112 Chicken IgY is an effective target mode in use for prophylaxis and therapy of pathogenic infection in poultry and other domestic animals

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Chicken IgY, the egg yolk antibodies (Ab) confer passive immunity to the offspring. The purpose of this study was to show the use of IgY as an anti-viral treatment in poultry and as an alternative to antibiotics for prophylaxis and therapy of domestic mammals and humans. Subcutaneous administration of anti-Newcastle disease virus (NDV) IgY to 1-day-old chick, resulted a protective antibody titers for at least 17 days of age. The combination of passive immunization on day 1 of age with attenuated live vaccination on day 10 led to high protective titers throughout the 41 days of growth period. Thus, full protection against NDV of all broilers in flock during their entire growth period was achieved by a vaccination regime that combines passive immunization and live vaccination. In an attempt to develop IgY against a broad spectrum of enteric E coli, a strain of E. coli that induced high level of antibodies to enteric E. coli was selected to immunize layers. The developed IgY effectively neutralized the tested enteric bacteria from poultry (O78), calve (k99) and piglet (K88). In a 3rd study, Y-Complex – a combination of IgY anti-mastitis bacteria and macrophage inducer was used and was effective in neutralizing mastitis bacteria in both in vitro and in vivo studies. Results of the described technologies using IgY may be applied against additional pathogens and diseases in animals.

Keywords: IgY, passive immunization, E. coli, NDV, poultry, mastitis

S3-0113 Molecular epidemiology of the H6 subtype influenza a viruses during 2009–2014 from poultry and wild birds in Guangxi, southern China

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Guangxi province is located in the Southern China region. There are several large-scale live bird markets (LBMs) and large number of small-scale poultry farms establishment located in this province. This area is considered as an “influenza epicenter” area. H6 viruses have been detected frequently from poultry and wild birds in Guangxi, although some of the H6 isolates data demonstrates has been disclosed. However, the molecular epidemiological characteristics of these viruses during 2009-2014 in Guangxi is still unknown. During this period, total 23 H6 viruses were isolated from duck, goose, chicken and pigeon and all the 8 gene segments of the isolates were sequenced by RT-PCR technology. The sequences were assembled and analyzed by molecular biology softwares. Epidemiology revealed that H6N2 and H6N6 viruses were the most frequently detected influenza subtypes in LBMs in Guangxi, but during 2009 -2011, there were at least three other different neuraminidase types of avian influenza A H6 viruses circulate. Moreover, we also isolated 3 H6 subtype avian influenza virus strains from pigeons which is the first report in China. Phylogenetic analysis indicated that the origin of these viral genes were comprehensive, many strains were reassorted with H5 or H9 and other subtypes viruses from surrounding regions or countries, even shared higher homology of nucleotides with human and swine influenza viruses. Two distinct hemagglutinin lineages were identified and they all underwent frequent reassortment with multiple virus subtypes from the natural gene pool, but few reassortants were persistent or prevalent. We also found that five different subtypes of H6 influenza viruses (H6N1, H6N2, H6N5, H6N6 and H6N8) cocirculated in Guangxi province, southern China, which form a significant part of the natural influenza virus reservoir in duck, goose, chicken, pigeon and significant viral reassortment is still ongoing in this species.

Keywords: H6 influenza viruses, poultry, wild bird, phylogenetic analysis, molecular evolution

S3- 0114 Expression and identification of $\sigma 3$ gene of avian reovirus in transgenic tobacco

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Present study focused on expression of Avian reovirus $\sigma 3$ gene in tobacco and their genicity reactions of the expression products. A pair of primers was designed according to the major antigen region of $\sigma 3$ gene derived from GenBank. A plant vector of pBI121- $\sigma 3$ constitutively expressed $\sigma 3$ gene was constructed. We speculated that the $\sigma 3$ gene may be expressed as a 61.6 ku green fluorescent fusion protein in tobacco. The pBI121- $\sigma 3$ vector was transferred into an Agrobacterium strain EHA105 by thermal activation method. After transformation of tobacco plants via Agrobacterium tumefaciens, the resistant plants were selected with kanamycin. The resistant plants were firstly analyzed by PCR, eight resistant plants containing $\sigma 3$ gene were obtained by PCR screening. Real-time fluorescence quantitative PCR were used to further estimate the copy number of $\sigma 3$ gene in positive plants along with endogenous RNR2 gene in tobacco as a reference gene. With a serial of dilutions, the standard curves of the cycle threshold relative to the log of each initial template copy of $\sigma 3$ and RNR2 genes were obtained. The transgenic copy number was obtained by comparing the initial template copy of $\sigma 3$ gene with that of RNR2. Among the five putative transgenic lines, five had four copy number, whereas the negative control had none. Western blot results showed that the $\sigma 3$ protein was successfully expressed, and the protein was specifically recognized by anti ARV positive serum. The research findings provide a basis for further analysis on plants as bio-reactors development and the production of $\sigma 3$ oral vaccines.

Keywords: avian reovirus, $\sigma 3$ gene, plant expression vector, tobacco

S3- 0115 Epidemiological surveillance of low pathogenic avian influenza virus from poultry in Guangxi province, Southern China in 2012–2015

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Low pathogenic avian influenza virus (LPAIV) usually causes mild disease or asymptomatic infection in poultry. However, some LPAIV strains cause severe infection with accompanied by other co-infection and can be transmitted to human. Genetic rearrangement and recombination events of LPAIV may generate a novel virus with increased virulence, posing a substantial risk to public health. Southern China is regarded as the world ‘ ‘influenza epicenter’ ’, due to a rash of outbreaks of influenza in recent years. In this study, we conducted an epidemiological survey of LPAIV at different live bird markets (LBMs) in Guangxi province, Southern China. From January 2012 to December 2015, we collected 3,522 cotton swab samples of larynx, trachea and cloaca every month from the poultry at LBMs in Guangxi. Virus isolation, hemagglutination inhibition (HI) assay, RT-PCR and sequencing were used to detect and subtype LPAIV in the collected samples. Of the 3,522 samples, 756 samples (21.5%) were LPAIV positive, including 171/1245 (13.7%) in chicken and 585/2277 (25.7%) in duck. The identified LPAIV were H1N2, H3N2, H3N6, H3N8, H4N2, H4N6, H6N1, H6N2, H6N5, H6N6, H6N8, H9N2 and H9N8, which are combinations of seven HA subtypes (H1, H3, H4, H6, H9, H10 and H11) and five NA subtypes (N1, N2, N5, N6 and N8). The H3 and H9 subtypes were predominant in the identified LPAIVs. Among the positive samples, at least 29 types of mixed infection of different HA subtypes were identified, and the mixed infections may provide opportunities for genetic recombination. Our results suggest that the LPAIV epidemiology in poultry in the Guangxi province in southern China is complicated and highlights the need for further epidemiological and genetic studies of LPAIV in this area.

Keywords: avian influenza virus, epidemiological, surveillance

S3-0116 Proteomic analysis of vero cells infected by avian reovirus virulent strain and attenuated vaccine strain

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To discover protein expression changes in infected vero cells with avian reovirus (ARV) virulent strain S1133 and attenuated vaccine strain aS1133, respectively, two dimensional gel electrophoresis (2-DE) and mass spectroscopy (MS) technologies were utilized to separate and identify the total proteins of vero cells inoculated with ARV S1133 or aS1133 as well as control cells at desired time points post infection. The identification results showed that total 44 protein dots were identified including α -enolase, Prx-4, hnRNPs, tubulin, protein phosphatase, transcription elongation factor. These differentially expressed proteins were involved in various biological functions, including signaling transduction, cytoskeleton, metabolism, protein disfolding and cell proliferation. The results of quantitative RT-PCR validated the mRNA expression of α -enolase, Prx-4, hnRNPs and tubulin were in agreement with that of proteomic analysis. The present data highlighted protein expression differences in infected cells with ARV virulent strain and attenuated vaccine strain, which would contribute to further elucidate the interaction between ARV and hosts.

Keywords: avian reovirus, virulent strain, attenuated vaccine strain, vero cells, differentially expressed proteins

S3-0117 The application of marketing methodology to the advocacy of on-farm biosecurity: Targeted strategies

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This study proposes a two-stage application of how social science methodology, namely cluster analysis and targeted strategies, can be applied to assist the advocacy of on-farm biosecurity. A semi-structured survey of 303 broiler farmers was conducted in Taiwan to collect farm biosecurity information. It was found that there was attitude-behaviour inconsistency which resulted in compromised biosecurity practices at the farm level. Using categorical principal components analysis and two-stage cluster analysis, two types of cluster were identified: (1) attitude clusters based on 15 variables indicated that despite recognising the importance of biosecurity measures, these farmers tended not to translate their knowledge into the relevant biosecurity actions; (2) behaviour clusters characterised by 30 variables represented that farmers who, despite not having a better attitude towards the importance of biosecurity, still took desirable biosecurity actions. Following this analysis, the study applied the concept of target strategies of consumer marketing to veterinary medicine for the advocacy of on-farm biosecurity by identifying five target clusters based on individual farmers' biosecurity attitudes and behaviours. Based on the clusters, relevant target strategies were planned, including targeted communication, resource supply, knowledge delivering, policy instruments and enforcement activities. These findings suggest a failure of effective policy implementation as a result of the government placing most effort on providing knowledge (based on the knowledge-attitude-behaviour model) and enforcement of policy (based on the counter-attitudinal advocacy model). Inappropriate application of behavioural change theory will not result in the desired biosecurity behaviours amongst many farmers and may instead induce cognitive dissonance amongst groups of farmers. Targeted strategies will be useful for the development of biosecurity policies in Taiwan's poultry farms.

Keywords: biosecurity attitude, biosecurity behaviour, broiler, cluster analysis, targeted strategies.

S3-0118 The impact of social structure on biosecurity practices in poultry farm: A qualitative study of farmers' perspectives in Taiwan

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The study aims to identify factors related to farmers' adoption of appropriate measures for on-farm disease management. An interview guide consisting of simple and short open-ended questions was constructed to conduct face-to-face interviews with poultry farmers in Taiwan; 25 farmers with more than 10 years' experience participated in the research. The study explored farmers' attitudes to on-farm biosecurity covering topics related to farm type, farmer's experience in farm management, biosecurity, resources, and physical farm environment; the data were analysed by qualitative content analysis. The study revealed the influence of macro socio-economic conditions, which have considerable impacts on disease control, the industry, and farm management. The study further identified challenges associated with disease management and farmers' strategies for dealing with these. In addition, farmers' suggestions regarding policy were also explored. A social ecological model was suggested to explain the complexity of social factors influencing on-farm biosecurity practices with multilevel social structure, including individual, interperson, society, organization and government levels. In the study, the following areas of deficiency perceived by farmers were revealed: (1) There is a misunderstanding regarding the effectiveness of biosecurity; (2) There are difficulties in changing farmers' current practices; (3) Farmers lack trust in the government; (4) Farmers believe that researchers rarely provide solutions to practical problems; (5) Farmers complain about the impracticality of current regulations; (6) Governments might not be aware of the gaps of obtaining true on-farm disease status information. These findings will be useful for informing the development of effective biosecurity policies for Taiwan's poultry farms.

Keywords: biosecurity, factors, perspective, poultry, qualitative study.

S3-0119 Influence of lipopolysaccharides (LPS) on bone physical properties and expression of bone-formation related genes

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High rearing density make chickens prone to suffer immune challenges. During immune challenge, the response of bone formation remains to be elucidated. In this study, we examined the effects of LPS on bone physical properties and expression of bone-formation related genes in laying-type chicks. One-week-old chicks were randomly divided into three groups and subjected to the following treatments respectively: injected subcutaneously over calvariae with LPS at the dose of 5mg/kg body weight(B group) and 15mg/kg body weight(C group) or sham injected with 0.9% NaCl solution (control, A group). The results showed that tibiae length, diameter and quality index were not influenced by LPS, femur length and diameter were also not influenced by LPS, but femur quality were reduced by LPS ($p=0.01$). In calvariae, the mRNA levels of IL-8 and TNF α were increased significantly by LPS, bone formation gene, Runx2, osteocalcin, osteopontin, Dmp1 were decreased significantly in both B and C group, but collagen I, Mepe were decreased significantly only in C group. Following changes happened in mRNA expression of following genes in tibiae, the IL-8 was increased significantly in both B and C group, but Dmp1, Mepe, Phex, Runx2 were decreased significantly only in C group, and TNF α , osteocalcin, osteopontin, collagen I were not changed significantly. In conclusion, LPS induced immune stress inhibits bone development and down-regulated mRNA expression of genes related to bone formation in calvariae and tibiae in a dose dependent manner.

Keywords: immune stress, LPS, bone formation

S3- 0120 Co- infection of broilers with Escherichia coli (O78) and avian influenza virus (H9N2)

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Escherichia coli (E.coli) is an important pathogen of domestic poultry and is prevalent in commercial poultry. Low pathogenic avian influenza virus (LPAIV; H9N2) infections are emerging respiratory problems in poultry industry, causing huge economic losses especially in the presence of other co-infecting pathogens. However, the effect of LPAIV on E.coli infection is not well understood. The aim of the present study was to investigate the infection of LPAIV H9N2 (A/chicken/Pakistan/UDL- 01/08) in chickens challenged with E.coli (O78:K80). Experimental broiler chicken were divided into four groups and challenged with an E.coli strain or LPAIV (H9N2) or both. The four experimental groups were identified as follows: negative control, E.coli, AI, and E.coli plus AI. Infected birds showed clinical signs of differing severity, with the most prominent disease signs appearing in birds of the E.coli plus AI group. Moreover, birds in E.coli plus AI group showed significant decrease in weight, enhanced macroscopic and microscopic pathological lesions. Specifically, the survival rate was 60%, 90%, and 100% in birds inoculated with E. coli+H9N2 virus, E.coli and control negative or H9N2 virus alone, respectively. The results showed that experimental co-infection of E.coli and LPAIV increased the severity of clinical signs, mortality rate and gross lesions and suggest that E.coli infection can induce higher economic losses and mortality if H9N2 LPAIV is also present. The HI titer against LPAIV infection in the co-infected group was significantly higher than the HI titer of AI group, which may indicate that E.coli could promote the propagation of H9N2 LPAIV or stimulate the immune response. The present study revealed that co-infection with LPAIV enhanced pathogenesis of E.coli infection significantly and indicates that LPAIV can act as a complicating factor in E.coli infections

Keywords: Escherichia coli, low pathogenic avian influenza, co-infection, pathology, broiler

S3-0121 Purification of avian leukosis CAU No.3 Layer

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This study was carried out to purify avian leukosis of CAU (China Agricultural University) No.3 Layer in our company. Avian leukosis virus (ALV) p27 antigen was detected with ELISA kits and ALV- AB/J antibody was accessorially detected. Meanwhile, cell culture and PCR were used to isolate, culture and identify ALV from suspicious samples. Purification procedures were as follows: the whole pedigree would be eliminated if ALV p27 was detected from the meconium of 1-d chicken. Ten percent of Chickens (6-10 weeks) were sampled to evaluate vertical transmission. Before laying eggs and in the first month before breeding, the whole groups were detected; Before and during semen collection period, the cocks were detected. The ALV positive ones were eliminated. Between 2008 and 2015, five matching strains (A, B, C, D and W) of CAU No.3 Layer were purified during seven generations. ALV positive rates of A, B, C, D and W strain (cocks and hens) were decreased from 3.33% (16/480) and 3.44% (95/2758) to 0.00% (0/510) and 0.18% (5/2846), from 2.59% (13/502) and 4.34% (131/3021) to 0.19% (1/521) and 0.13% (4/3124), from 9.04% (45/498) and 4.70% (131/2789) to 0.19% (1/534) and 0.20% (6/2976), from 8.32% (50/601) and 5.23% (156/2984) to 0.00% (0/548) and 0.14% (4/2935), from 9.48% (55/580) and 4.61% (132/2865) to 0.00% (0/564) and 0.14% (4/2814), respectively. Purification of avian leukosis in CAU No.3 Layer has achieved significant effect through detection and comprehensive environment controlling over seven generations. Currently, no clinical cases of avian leukosis were found in 80 million commercial layers.

Keywords: CAU No.3 layer, avian leukosis, ALV p27

S3-0122 Effect of supplementing organic acid- based feed additives on Salmonella prevalence, gut microbiota and growth performance of broiler chickens challenged with Salmonella typhimurium

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Salmonellosis in humans has been often linked with consumption of eggs and meat from poultry and swine. This study compared the efficacy of a multi-hurdle product Fysal Fit-4 (blend of organic acids, medium chain fatty acids, butyrate & mannobiose) with an addition of an organic acid mixture (OA, blend of formic acid, propionic acid and acetic acid), or coated sodium butyrate (Butyrate) in reducing *S. typhimurium* in broiler chickens. 880 day-old chicks were allocated to four dietary treatments, with 10 floor pens (22 birds/pen) per treatment. The trial consisted of Phases 1 (day 1 to 18) and 2 (day 18 to 42). The treatments included: (1) Control, (2) + Fysal Fit-4 (3 and 1 kg/tonne in Phases 1 & 2 respectively), (3) + Fysal Fit-4 (1 kg/tonne in Phase 1) + OA (1.5 kg/tonne in Phase 2), and (4) + Butyrate (1 kg/tonne). At days 8 & 9, all birds were orally challenged with 1 ml of a *S. typhimurium* inoculum (10^8 - 10^9 cfu/ml). At day 28, 35 and 42, two birds per pen were sacrificed to determine the *S. typhimurium* counts in the ceca. Cecal bacterial 16S rDNA sequencing was conducted to determine the impact of the supplements on gut microbiota by using the Illumina technology. The results showed that all supplements reduced the Salmonella counts compared with the control group at day 42, with the most reduction achieved by Fysal Fit-4 (-0.6 log), followed by Fysal Fit-4 + OA (-0.52 log). Fysal Fit-4 also improved the feed efficiency of the broilers ($P < 0.0001$) and reduced the mortality numerically. According to the microbiota analysis, Fysal Fit-4 addition also resulted in the highest Firmicutes to Bacteroidetes ratio in ceca among all the treatments. In conclusion, Fysal Fit-4 reduced the Salmonella counts in the ceca, improved the feed efficiency, and impacted the gut microbiota of the broiler chickens.

Keywords: broiler chickens, Salmonella, feed efficiency, microbiota

S3-0123 Ovodefensins: the relationship between structure and function

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Ovodefensins are a novel beta-defensin family of antimicrobial peptides which are expressed in the oviduct for secretion into the egg. They are related to avian defensins and contain a conserved glycine and the classic six cysteine motif of β -defensins in two forms: C-X5-C-X3-C-X11-C-X3-CC and C-X3-C-X3-C-X11-C-X4-CC. However they are shorter in length and differ in cysteine spacing compared to classical β -defensins. Using new search strategies the ovodefensin family now has 35 members but no representatives from classes out with aves and reptilia have been found. Analysis of their evolution shows that ovodefensins divide into 6 groups based on the intra cysteine amino acid spacing, representing a unique mechanism alongside traditional evolution of sequence. The groups have been used to base a nomenclature for the family. Recent structural analysis of chicken ovodefensin Gallus gallus OvoDA1 (gallin) revealed a five-stranded arrangement of beta-sheets supporting the hypothesis that ovodefensins form a structurally distinct subfamily of beta-defensins. Antimicrobial activity for three ovodefensins from chicken and duck was confirmed against both a gram negative laboratory adapted and pathogenic *E. coli* strain as well as a gram positive bacteria, *S. aureus*. However, activity varied greatly between peptides, with Gallus gallus OvoDA1 being the most potent, suggesting a link between structure and function. The divergent motif structure and sequence of ovodefensins present an interesting area of research for antimicrobial peptide design, specifically investigating how structure effects function. The discovery of new antimicrobial innate defence molecules and understanding their structure-function relationship may have potential application as poultry antimicrobials.

Keywords: antimicrobial, defensin, egg, evolution, oviduct, chicken

S3- 0124 Assessment of sanitation procedures on pathogen prevalence in commercial broiler barns in Ontario, Canada

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Biosecurity is paramount for reducing disease on farm premises. Our objective was to determine how pathogen loads are affected by sanitation procedures recommended by the poultry industry. Thirty-six commercial 2-level broiler barns (concrete lower level and wooden upper level) were conveniently enrolled. Each barn was dry cleaned (DRY), wet cleaned (WET), or disinfected (DIS) after flock removal using the protocol normally performed by the producer. Four randomly selected 1m² areas per floor were swabbed using D/E neutralizing sponges. Samples were collected at three time points: after litter removal (BASE); 2-days post sanitation (2D); and 6-days post sanitation (6D). Samples were submitted for *E. coli* quantification and presence of *Salmonella* spp. and *Clostridium perfringens*. *Escherichia coli* isolates were tested by PCR for resistance genes *qacEA1* and *sug(E)*. Preliminary results (first 17 barns) for concrete floors are shown. The mean *E. coli* count for the DRY, WET, and DIS groups, respectively were 14892, 26398, and 25258 cfu/g for BASE samples, 1869, 4401, and 343 for 2D samples, and 1449, 6444, and 385 for 6D samples. The proportion of samples positive for *C. perfringens* for the DRY, WET, and DIS groups, respectively were 7%, 0%, and 14% for BASE samples, 7%, 1%, and 1% for 2D samples, and 8%, 0%, and 3% for 6D samples. The proportion of samples positive for *Salmonella* for the DRY, WET, and DIS groups, respectively were 7%, 0%, and 12% for BASE samples, 7%, 1%, and 5% for 2D samples, and 5%, 2%, and 6% for 6D samples. Mixed regression models will be used to identify associations between presence/concentration of organisms and sanitation procedure (DRY, WET, DIS), flooring type (wood, concrete), rest period (BASE, 2D, 6D), and other factors (e.g. type of disinfectant, water temperature), and between disinfectant use and antimicrobial resistance. Results will be used by the Ontario broiler industry to update standards for cleaning and disinfecting barns.

Keywords: biosecurity, sanitation, cleaning and disinfection, quaternary ammonium, antimicrobial resistance

S3-0125 Antimicrobial resistance of *Clostridium perfringens* isolates obtained from commercial broiler chicken flocks in Ontario, Canada

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Clostridium perfringens is the causative agent of necrotic enteritis in chickens. Although the exact role of netB in disease development is not known, this gene is considered an important virulence factor in disease pathogenesis. Our objectives were to determine: 1) the antimicrobial susceptibility of *C. perfringens* isolates obtained from 231 randomly selected Ontario broiler flocks to 11 antimicrobials of importance to veterinary medicine; and 2) associations between resistance and on-farm biosecurity, management, antimicrobial use, and netB. Five pooled samples of cecal swabs from 15 birds/flock were anaerobically cultured for *C. perfringens*. Real-time PCR was used to test isolates for netB. Minimum inhibitory concentrations were determined using the microbroth dilution method. Multivariable generalized estimating equations were used to identify significant associations ($p \leq 0.05$). *Clostridium perfringens* was isolated from 181 flocks (78%). NetB was identified in 71 flocks (31%) and in 169 of 629 *C. perfringens*-positive isolates (27%). Isolates were resistant to bacitracin (82.2%), oxytetracycline (64.5%), erythromycin (62.3%), tetracycline (62.2%), ceftiofur (49.4%), clindamycin (21.1%), tylosin (18.8%), and penicillin (0.2%). Multi-class resistance was common (64% of isolates). In-feed bacitracin use was a risk factor for resistance of *C. perfringens* to bacitracin (OR=4.1), and in-feed tylosin use was a risk factor for resistance to tylosin (OR=6.6), clindamycin (OR=9.7), and erythromycin (OR=6.4). The presence of netB was protective against resistance to bacitracin (OR=0.3), clindamycin (OR=0.5), and erythromycin (OR=0.6), yet a risk factor for resistance to ceftiofur (OR=1.6), oxytetracycline (OR=4.1), and tetracycline (OR=7.5). Finding alternatives to the use of antimicrobials in the feed to prevent necrotic enteritis should continue to be a priority. Molecular studies are needed to understand relationships between netB and resistance to certain antimicrobials.

Keywords: *Clostridium perfringens*, netB gene, antimicrobial use, antimicrobial resistance, husbandry

S3-0126 Dietary supplementation of a multi-strain probiotic SYNLAB® II improves broiler intestinal health and immunity

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Probiotics are live microorganism that contribute to the health and balance of host intestinal tract and were demonstrated by many researches to be possible alternative to antibiotics as growth promoters. The objective of this study was to evaluate the probiotic characteristics in vitro and in vivo of a multistrain direct-fed microbials (SYNLAC® II) that composed of three strains of lactic acid bacteria (LAB), *Enterococcus faecium* EF08, *Lactobacillus plantarum* LP28, *Lactobacillus acidophilus* LAP5 and one strain of *Bacillus subtilis*. From the in vitro tests, combination of the three strains of LAB was demonstrated to have synergistic effect on their ability to adhere to Caco-2 cells and stimulation of IFN- γ secretion by chicks' peripheral blood mononuclear cell (PBMC). According to the in vitro data, we further study the effect of this multi-strain probiotic SYNLAB® II on broiler performance. A total of 80 one-day-old Arbor Acres chickens were randomly distributed into two groups with 4 replicate pens per treatment (10 birds/ replicate pen). The birds were fed basal diet or basal diet with a multi-strain probiotic SYNLAB® II at 106 CFU/g of the feed. From the results, the ileum villus height was higher and crypt depth was lower in SYNLAB® II group compared to control group ($P < 0.05$). The level of the immunoglobulin G of the serum was significantly enhanced in SYNLAB® II group ($P < 0.05$). However, there was no significant difference in feed conversion ratio ($P > 0.05$) between the two treatments. In conclusion, the multi-strain probiotics SYNLAB® II could improve the intestinal health, as well as enhance the immune response against infections.

Keywords: probiotic, IFN- γ , broiler, intestinal health

S3-0127 Characterization of H9N2 influenza viruses in South Korea, 2012-2014

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Since 2007, Korea has used inactivated H9N2 low pathogenic avian influenza (AI) vaccine to control the disease. Despite the long-term vaccination programs, H9N2 avian influenza viruses continue to persist in chicken populations, mainly in Korean native chickens of unvaccinated flocks and live bird markets. H9N2 influenza viruses were isolated from live bird market through the national AI surveillance program, 2012-2014. In phylogenetic analysis, the HA gene belonged to Y439-lineage, which was gradually evolved by point-mutation from MS96. In contrast, the NA gene was related to H6N2 or H3N2 in Korean domestic duck or wild birds. The NP gene like the HA gene was belonged to the MS96, whereas the remaining internal genes, PB2, PB1, PA, M and NS were genetically reassorted with various subtype AIVs of Korean wild bird and poultry. In mice, the eight of eleven H9N2 viruses were replicated in lung of mice to relatively low titers ($10^{0.9} - 3.1 \text{EID}_{50}/50\mu\text{l}$). However, none of group infected H9N2 viruses exhibited weight loss or clinical symptoms during the observation period. In SPF chickens, we evaluated the protective efficacy of a commercial vaccine, a pilot vaccine, and unvaccinated group against recently circulating H9N2 AIVs. Challenge experiments using four H9N2 isolates indicated a commercial vaccine did not prevent completely viral shedding and replication in swab samples and cecal tonsil although it covers the scope of vaccine efficacy against recently circulating H9N2 isolates. In conclusion, our study has demonstrated that the Korean H9N2 virus in live bird markets have circulated continually with genetic reassortment. In addition, this virus may have the potential to replicate and to directly infect mammals without prior adaptation. Therefore, our findings emphasize the need to expand influenza surveillance in poultry, especially live bird market.

Keywords: H9N2, live bird market, evolution, vaccine, South Korea

S3-0128 Serological survey on avian metapneumovirus infection in Anhui chicken flocks

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Avian metapneumovirus (aMPV) is one of the major causes of serious respiratory infections of poultry, and the infections are often associated with swollen head syndrome (SHS) in chickens. To investigate infection of aMPV in chicken flocks in Anhui province, antibodies to the virus were tested based on 296 serum samples that collected from 7 different breeds (strains) of 9 companies in Hefei, Bozhou, Dingyuan, Shucheng and other areas. The results showed that all farms were infected by aMPV. The antibody-positive rates of tested serum samples were as high as 100% in some farms, and the lowest rate was 20%. All chicken breeds (strains) were infected by aMPV in the study. The positive rate of meat-type partridge chickens with green feet was the highest, followed by Cobb broiler chickens, Hyline laying hens, Yuehuang hens, Huannan partridge chickens, yellow local chickens and Xinguang partridge chickens. Moreover, the infected chickens accounted for 88.7% in laying hens, the positive rate was higher than those in broiler chickens and dual-purpose chickens. The antibody-positive rates were also high in both hens and cocks. These results indicated that aMPV infection of chicken flocks was prevalent in Anhui province, and all the infection were serious among chicken flocks of different areas, breeds, function and gender. Therefore preventive measurements should be carried out based on the infection status as soon as possible.

Keywords: chicken, avian metapneumovirus, serological survey

S3-0130 Complete genome sequence of avian paramyxovirus type 4 isolated from domestic duck in South Korea

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Avian paramyxoviruses are enveloped, negative sense single-stranded RNA viruses belongs to the genera Avulavirus of the family Paramyxoviridae. To date, 12 different serotypes and APMV-4 has frequently isolating from wild waterfowl around the world and that can spill over to poultry. So far, totally 6 full-length and 24 partial genome analysis of 26 APMV-4 isolates reported at Genbank include 1 full-length and 11 partial genome analysis of Korean strains that all isolated from wild duck. This study represents the first full genome sequence of APMV-4 isolated from domestic mallard duck at Live Bird Market (LBM) of South Korea. The 15,054 nt in length and ordered as 3'-N-P-M-F-HN-L-5' complete genomic sequence determined by next generation sequencing using Ion Torrent sequencer platform. The mapping and annotation of total 5,996 reads of which 120,956 nt short reads alignments were assembled by Geneious 8.1.8 software with reference genome APMV-4/KR/YJ/06. Comparison of full genomic sequences of APMV-4 strains indicated APMV-4/Mallard/LBM/Korea/N19/2012 has 94.6% sequence identity with APMV-4/mallard/Belgium/15129/07, 94.3% with APMV-4/KR/YJ/06, 93.5% with APMV4/duck/China/G302/2012, 93.4% with APMV-4/Egyptian goose/South Africa/N1468/10, 92.7% with APMV-4/duck/Hongkong/D3/75 and 85.8% with APMV-4/duck/Delaware/549227/2010. Consequently, we compared the replication capacity, transmissibility, and pathogenicity of two APMV-4 Korean strains isolated from wild and domestic birds in 1 day-old ducks. In the experiment, both viruses was detected in the viral shedding samples in the oropharyngeal and cloacal swabs but did not cause any clinical signs. Although the replicative capacity and transmissibility of APMV-4/Mallard/LBM/Korea/N19/2012 in ducks was higher than APMV-4/Mandarin duck/KR/004/2013 in these studies, The result suggest APMV-4/Mallard/LBM/Korea/N19/2012 strain is possible candidate for vaccine vector and further characterization of this strain is required.

Keywords: avian paramyxoviruses, APMV-4, next generation sequencing, Korea, live bird market

S3- 0131 Identifying the genes involved in iron metabolism in *R. anatipestifer* CH- 1 through RNA-Seq- Based analysis in iron- limited medium

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One of the important elements for most bacterial growth is iron, whose bioavailability in host is limited. *R. anatipestifer*, an important pathogen for duck, absolutely need iron to alive. However, the genes involved in iron metabolism and the mechanisms of iron transport are largely unknown. In this study, we investigated the transcriptomic effects of iron limitation on *R. anatipestifer* CH- 1 using RNA- Seq technique. Analysis of the data revealed that encoding functions related to iron homeostasis, including a number of putative TonB-dependent receptor systems, Feo system, a gene cluster related to starch utilization, as well as genes encoding for hypothetical protein were significantly up-regulated in response to iron limitation. Knockout one of hypothetical proteins damaged seriously iron uptake of *R. anatipestifer* CH- 1. It suggested that RNA-Seq-based analysis in iron-limited medium is a effective and fast method to find the genes involved in iron uptake in *R. anatipestifer* CH-1.

Keywords: *R. anatipestifer* , iron utilization, RNA-Seq

S3- 0133 Development of eggshell membranes – based wound healing products

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The chicken eggshell membranes (ESM) are cross-linked fibrous proteins which has been used for the treatment of wound / burn injuries in Chinese traditional medicine. The objective of this study was to characterize the ESM protein constituents using proteomics and bioinformatics analysis, and to investigate an ESM-based product in an in vivo wound healing model. The inventory of ESM proteins and DAVID bioinformatics analysis identified proteins that have potential roles in different wound healing phases. Antimicrobial proteins including ovotransferrin, lysozyme, avidin, retbindin, ovocalyxin-32, ovalbumin-related protein X and cystatin C, as well as protease inhibitors that are critical for correction of the chronic wound milieu such as ovostatin and similar to kunitz-like protease inhibitor, were detected. In addition, proteins that promote angiogenesis and re-epithelialization including lysyl oxidase-like 2 along with proteins that act as a matrix for fibroblast migration including collagen X, were identified. The mouse splinting model was utilized to evaluate ESM promotion of wound healing. A 6-mm rounded, full-thickness excisional wound was created on each side of the dorsal midline. Different concentrations of ESM powder were applied as a suspension in PBS, while the control side received vehicle. The kinetics of wound area closure was assessed by image analysis over 21 days. ESM significantly improved wound healing at early time points compared to the vehicle-treated control wound. No adverse effects from ESM application were observed. Fibrous biopolymers such as collagen and ECM preparations are known to assist wound healing, and these materials are somewhat structurally analogous to ESM. It is, therefore, likely that ESM functions in wound healing to a large extent due to its physical structure. However, the presence of bioactive proteins within the matrix may assist and in part account for its wound healing activity.

Keywords: eggshell membranes, wound healing, collagens, proteomics

S3- 0134 Comparative proteomics analysis of egg white, egg yolk, and eggshell membranes following acid or base hydrolysis

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The hen's egg contains a great variety of nutrients that sustain both life and embryonic growth. The eggshell membranes (ESM) is a fibrous meshwork of highly cross-linked proteins, which has made proteomic identification of its constituents technically very difficult. The objective of this study was to compare proteomic profiles for standard sample preparation (soluble in LDS, lithium dodecyl sulfate) to the harsher conditions of acid (1.25 M 3-mercaptopropionic acid/10% acetic acid) or base (5% NaOH/DMSO) hydrolysis. Egg white and yolk have well-known protein constituents and provided validated results for comparison to acidic and basic solubilization of ESM. Proteomics analysis was carried out to identify the resultant proteins after each solubilization strategy; data from all extraction conditions was compiled to create a complete protein inventory. We identified 98, 95, and 46 proteins in control (LDS-soluble) samples of egg white, egg yolk, and ESM, respectively. Twenty five (25) of these identified proteins were common to the three egg compartments including apovitellenin 1, vitellogenin 1, ovalbumin, ovotransferrin, lysozyme C, clusterin and retbindin. The extreme conditions of acid or base hydrolysis resulted in identification of fewer proteins. Eighteen (18), 37, and 19 proteins were identified after acidic solubilization, and 13, 17, and 14 were identified after base hydrolysis of egg white, yolk and ESM, respectively. A number of proteins were identified only after a harsh solubilization condition. In particular, members of the CREMP family were uniquely identified in ESM after acid hydrolysis only. In conclusion, extraction of insoluble proteins under conditions of acid or base hydrolysis interferes with the subsequent proteomics analysis; however, these harsh conditions allow additional proteins to be identified for the final protein inventory.

Keywords: proteomics analysis, solubilization, eggshell membranes, egg white, egg yolk

S3- 0135 Histones from chicken erythrocytes demonstrate broad-spectrum antimicrobial activity against planktonic bacteria

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The overuse and misuse of antibiotics has led to the increasing prevalence of antibiotic resistant bacteria, which are a global health challenge. Therefore, development of alternatives to traditional antibiotics is of great importance. Cationic antimicrobial peptides (CAMPs) are components of the innate immune system. Histones, commonly known for their role in DNA stabilization and packaging, share all of the essential antimicrobial traits of CAMPs, and could be a promising alternative to antibiotics. In this study, histones were extracted and purified from chicken erythrocytes by acid extraction; the purity was verified by LC/MS/MS proteomics analysis. A mobility shift assay was developed to investigate histone binding to bacterial LPS and LTA. The potential for histone toxicity towards mammalian cells was investigated with a hemolytic assay based on rat erythrocytes. The minimum inhibitory concentrations (MIC) of the purified histones were determined against various Gram+ and Gram-planktonic bacteria. The proteomics analysis revealed that the histone mixture contained H1 (9.7%), H2A (15%), H2B (19%), H3 (24%), H4 (14.6%) and H5 (18%). The mobility shift assay showed that histones can target pathogens via their membrane LPS or LTA components; however, the histone mixture did not damage mammalian red blood cell membranes. The Gram+ bacteria (*S. aureus*, MRSA, *B. subtilis* and *E. faecalis*) had MICs of 6 ± 1 , 8 ± 2 , 3 ± 1 and 700 ± 100 $\mu\text{g/mL}$, respectively. The Gram- bacteria (*E. coli*, *P. aeruginosa* and *S. typhimurium*) had MICs of 21 ± 3 , 3.6 ± 0.4 and 5 ± 1 $\mu\text{g/mL}$, respectively. These results show that histones purified from chicken erythrocytes have broad-spectrum antimicrobial activity since they inhibit the growth of both Gram+ and Gram-planktonic bacteria. Overall, this study demonstrates that histones could be promising candidates in the development of novel antibiotics and add value to avian blood, which is a significant waste product in the poultry industry.

Keywords: histones, cationic antimicrobial peptides, antibiotic resistance, chicken erythrocytes, pathogenic bacteria

S3- 0136 Antimicrobial effects of chicken erythrocyte histones against MRSA and methicillin- susceptible Staphylococcus aureus biofilms

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Antibiotic-resistant biofilms are implicated in cystic fibrosis and otitis media, as well as chronic wounds and infected medical devices. Therefore, novel ways to eradicate bacterial biofilms are essential for treating a variety of infections. The main objective of this work was to investigate antimicrobial activity of histones purified from chicken erythrocytes against Staphylococcus aureus and Methicillin-resistant S. aureus (MRSA) biofilms. The histone mixture was purified by acid extraction from erythrocytes of White Rock chickens, freeze-dried and dissolved in water. The antimicrobial effect was tested using the minimum biofilm eradication concentration (MBEC) plates that contain pegs serving as a surface for biofilm growth. Biofilms were grown for 24h, and then exposed to histones. The attached bacteria were shaken off the pegs by sonication; growth in LB was monitored over 24h using EON microplate spectrophotometer and Gen5 data analysis software. The results showed that the MRSA biofilm had an MBEC of $21 \pm 5 \mu\text{g/ml}$ compared to a minimum inhibitory concentration (MIC) of $7 \pm 2 \mu\text{g/ml}$ for the planktonic MRSA. The methicillin-susceptible S. aureus biofilm had an MBEC of $23 \pm 5 \mu\text{g/ml}$ compared to $6 \pm 2 \mu\text{g/ml}$ for the planktonic bacteria. The MRSA and S. aureus biofilms have an identical histone MBEC, and planktonic forms of both strains were equally susceptible to histones; therefore, histones have a different mechanism of action than methicillin. The bacteriolytic properties of histones were observed with LIVE/DEAD viability stain. There was a steady increase in red fluorescence (damaged membranes) at histone concentrations $>16 \mu\text{g/ml}$ for S. aureus, while MRSA showed an increase at $>32 \mu\text{g/ml}$. Bacterial upregulation of genes that reduce their surface charge in response to histone challenge was monitored by qRT-PCR. Overall, this study demonstrates that histones derived from chicken erythrocytes provide a novel method of eradicating MRSA biofilms.

Keywords: antimicrobial peptides, histone, biofilm, chicken erythrocytes, MRSA

S3-0137 Antimicrobial susceptibility test for avian botulism pathogen

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This describes a method and a result of antimicrobial susceptibility test for Clostridium botulinum toxin type C/D. According to British Society for Antimicrobial Chemotherapy manual, total 20 antibiotics include bacitracin and penicillin was tested for 10 bacterial strains. Although, there is no reference interval for this pathogen, it was suspected to have resistance to trimethoprim-sulfamethoxazole, tri-sulfa and cycloserine because there is no clear zone. On the other hands, diameters of bacitracin and penicillin are 26.6mm and 24.9mm. Resistance drugs would use as selective agent for selective media. And the other can use in avian botulism outbreaks.

Keywords: clostridium botulinum, botulism, antimicrobial susceptibility test

S3-0138 Broad protection against infectious bursal disease of chickens

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Infectious bursal disease (IBD) can be caused by classic virulent, very virulent and variant sub-clinical viruses. Both virus types, classic virulent or variant, cause immune suppression which can lead to secondary infections and vaccine failures. Vaccination is the main method to ensure protection against all pathotypes of IBDV. Herpesvirus of turkey HVT vector vaccines of IBDV combine the advantages of live modified vaccines against IBDV without their safety problems and allow early vaccination at the hatchery by day-old or in ovo injection against both Marek's disease and IBDV in the presence of maternally-derived antibodies. Clinical protection against IBDV is measured by protection of the target tissue of the virus, the bursa of Fabricius, a primary lymphoid organ of the chicken. Wide clinical protection against the different pathotypes of IBDV is needed, i.e., against classic virulent and very virulent IBDV, mostly found in Asia and Europe, parts of South America, as well as against variant virus challenges, mostly found in North America, and spreading elsewhere, with some recent evidence in South America and Asia mostly. The classic IBDV-based vaccine construct, from the classic IBDV Faragher 52/70 strain, ensures production of the appropriate antigen to stimulate the immunity of poultry against all tested pathotypes of IBDV from all continents in which IBDV is circulating and including the most pathogenic strains for example from China. Vaccination results in IBDV VP2 transgenic protein generation, and therefore stimulation of the immunity of the host against these specific epitopes. Observation of wide clinical protection against all tested pathotypes of IBDV around the world is linked to the nature of the VP2 insert in the HVT vector vaccine coming from the Faragher 52/70 IBDV virus pathotype. The immunogenicity of the VP2 antigen produced is the key feature for obtaining wide protection against IBDV.

Keywords: Infectious bursal disease, vaccination, protection, pathotypes

S3- 0139 Effect of Bacillus direct-fed- microbial and natural plant extract products on necrotic enteritis and performance in broilers

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The inclusion of ionophores in one or more phases of feeding is often used to alleviate necrotic enteritis (NE) or subclinical enteritis in coccidiosis vaccination programs in the US (called bioshuttle programs); however, ionophores, considered as antibiotics in the US, are not accepted in antibiotic free (ABF) programs. Without using ionophores and antibiotic growth promotants (AGP's) in the feed, NE has become a major concern for poultry producers. Since the market trend of ABF, more and more natural products have been applied in the field to improve performance. Two different trials were conducted to evaluate the potential of antibiotic-free products (Bacillus licheniformis (BL), Bacillus subtilis (BS), dietary capsicum- turmeric oleoresins (CT), amprolium, and different combinations thereof) to improve performance and ameliorate the effects of NE in broiler chickens. All of birds in the two trials were sprayed with a full dose of coccidiosis vaccine and raised to 42 days (Trial 1) and 41 days (Trial 2). Birds were challenged in Trial 2 with 108 Clostridium perfringens for 3 consecutive days starting at day 18 and necropsied one day after the challenge to evaluate NE lesions. Body weight and feed conversion data were recorded in all trials. The results from Trial 1 demonstrated that the adjusted feed conversion ratio at day 42 of the BL and BS with or without amprolium 72.6 g/ton in the grower feed groups was significantly ($P < 0.05$) lower than the non-additive control group. Trial 2 showed that the BL, BS and CT product combination with amprolium 113g/ton (at 15-35 days) was the most beneficial for managing NE, followed by the same combination of products without amprolium. Under the conditions of these trials, it is concluded that BL, BS, and CT products are reliable solutions for the management of necrotic enteritis and improving performance in broilers vaccinated for coccidiosis.

Keywords: coccidiosis, necrotic enteritis, Bacillus, probiotic, essential oil

S3-0140 Efficacy of commercial infectious coryza vaccines against *Avibacterium paragallinarum* serovar A, B and C infection

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The objective of this study was to compare the efficacy of Infectious Coryza vaccines available in Thailand against local strains infection. Four commercial Infectious Coryza vaccines in Thailand were examined for protection rate against Thai field isolates serovar A, B, and C. Three Hundred and Thirty Six, male, layer were divided into 25 groups. Groups 1-18 were vaccinated twice at 9 and 13 weeks old. Groups 18-24 served as positive controls and group 25 served as a negative control. Then, groups 1-24 were challenged with *Avibacterium paragallinarum* at 15 weeks old. The result showed that vaccines 1 and 2 were 100% protection the birds against serovar A, B, and C Thai field isolates. Vaccine 3 were 100% protection against serovar B and C, while, Vaccine 4 were 100% protected against only serovar B. No adverse reaction of vaccines was observed in any group. This study revealed that the protection rate of Infectious Coryza vaccines were depended on the strains isolated from each country.

Keywords: *Avibacterium paragalm*, infectious coryza, vaccine efficacy, cross protection

S3-0141 Optimization of the vaccination schedule of the recombinant genotype VII strain inactivated NDV vaccine in layer chickens

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Newcastle disease (ND) is a devastating disease of poultry in China. The intensive vaccination schedules with very high frequency are usually implemented for the control of ND in layer breeders. In this study, we tried to optimize the ND vaccination schedule. Four hundred one-day-old healthy Jing Brown layer breeders were randomly divided into four groups. Chickens in group A were immunized with live and inactivated vaccine strain LaSota on day 7 and 21, respectively. The chickens in group B, C and D were immunized with LaSota-based live vaccine on day 7, 15 and 21, respectively, after spraying with the same live vaccine at hatching. On day 21, the chickens in these three groups received the third immunization with the A-VII-based inactivated vaccine. The results of hemagglutination inhibition (HI) assay showed that A-VII-based inactivated vaccine can induce HI antibody quickly and generated 2-3 log₂ higher HI titer than La Sota. Additionally, the protective efficacy result showed that chickens immunized with the A-VII strain-based vaccine had better clinical protection and 10-100 times lower virus shedding after virulent circulating genotype VIId virus challenge when compared with chickens vaccinated with La Sota. However, the protective efficacy between group B, C and D had no significant difference. Furthermore, trachea samples were collected from three chickens once a week post-secondary live-vaccine immunization. The antibody result indicated that mucosal immunity may also play an important role in efficacy evaluation. In summary, the immunization procedure that chickens are vaccinated at hatching with live vaccine, and on day 21 concurrently with live and inactivated vaccine is recommended, especially for those large-scale breeding groups with good sanitary condition of raising environments. The NDV genotype VII strain inactivated vaccine is safe, highly efficacious and could be chosen as a promising vaccine for the control of current ND epidemic in China.

Keywords: Newcastle disease, genotype VII vaccine, vaccination schedule, mucosal immunity

S3- 0144 Isolation and characterization of *Avibacterium paragallinarum* from quails in Yogyakarta, Indonesia

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Infectious coryza/snot is one of the upper respiratory diseases affecting chicken and birds, including quails that is caused by *Avibacterium paragallinarum*. This disease presents in both acute and chronic condition. The symptoms of snot disease is facial swelling, malodorous nasal discharge, and lacrimation. The aim of this study is to isolate and identify the *Avibacterium paragallinarum* of snot disease in quails in the field. There were nine quails from quail farm in Yogyakarta with obvious snot disease symptoms used in this study. The nasal swab was performed and directly streaked in onto chocolate agar incubated at 37°C for 24 hours in a candle jar. The observation of the morphology of the suspected colony, Gram staining, and biochemistry tests (catalase test, oxidase, urease, peptone, and carbohydrate fermentation such as maltose, mannitol, lactose, and sorbitol). The *Staphylococcus* sp. was added into the chocolate plate to show the satellite colony. The second step was performing sensitivity tests towards several antibiotics from five *Avibacterium paragallinarum* isolates that were cultured on Mueller- Hinton Agar using antibiotic discs, then incubated using candle jar at 37°C for 24 hours. Result study found that five isolates from nine suspected isolates 55.5% were *Avibacterium paragallinarum*. The isolates from quails did not depend on the NAD (NAD-independent). *Avibacterium paragallinarum* were 100% sensitive toward Amoxicillin and Ampicillin; and 100% resistant toward amikacin, Erythromycin, Gentamycin, and Tetracycline; 80% resistant toward Kanamycin and Trimethoprim; 60% resistant toward Chloramphenicol.

Keywords: infectious coryza, *Avibacterium paragallinarum*, NAD-independent, sensitivity test

S3- 0145 Species identification of *Campylobacter* spp and close relatives using a microsphere-PCR method

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The genus *Campylobacter* is one of the most common causes of bacteria foodborne disease worldwide with *C. jejuni* and *C. coli* accounting for more than 90% of the cases associated with this genus. Importantly, other close relatives *C. lari*, *C. upsaliensis*, *Helicobacter pullorum* and *Arcobacter butzleri* are also either associated with human illness, found in the same environments or regarded as emerging foodborne pathogens. For routine diagnostic and epidemiological monitoring purposes, we have developed a multiplex microsphere-PCR method targeting all 6 species. To avoid false negatives and problems associated with horizontal gene transfer, two species-specific genes were selected for each species, making a 12-plex PCR reaction. The signal collection was achieved by measuring the consumption of primers via 12 regions of Luminex microsphere. DNA from caecal samples was prepared by QIAamp DNA stool kit and repeated bead beating plus column. DNA from pure cultures was isolated using Qiagen Generation capture column kit. The total turnaround time for PCR and signal reading on the flow cytometer is 4 hours. Both in silico analysis and use of pure cultures of confirmed identity, the specificity of the assay has been confirmed. All 12 genes of 6 species have been specifically detected. The assay also correctly detected the various species when mixed pure cultures (upto six species tested) were used. Direct detection from caecal sample has also been achieved, although the sensitivity is lower than with pure cultures. The current version of the assay is suitable for use with pure and mixed cultures. However, improvements in DNA isolation methodology and manipulation of the assay conditions are needed to allow confident use directly on caecal material.

Keywords: *Campylobacter*, PCR, microsphere, Luminex

S3- 0146 Antiviral activity analysis of the Ostrich 2' – 5' Oligoadenylate Synthetase-Like (OsOAS-L)

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Up-to-date the Flaviviruses infection in avian taxa are not clearly defined. Several reports demonstrated that many viruses belonging to Flaviviridae cause diseases in poultry species such as West Nile Virus (WNV) and Israel Turkey Meningoencephalitis Virus and others. In mammals, the oligoadenylate synthetase gene family (OAS) was extensively investigated, and associated with resistance/susceptibility to Flavivirus infection, such as WNV, Dengue Virus (DV), and Tick Borne Encephalitis Virus (TBEV). However, the avian OAS proteins were rarely studied, although the OAS gene, one of the most important interferon-stimulated genes, contributes to the host antiviral response upon the Flavivirus infection. In our previous study, we confirmed that the chicken OAS-L protein (ChOAS-L) expressed the enzymatic activity (the classical OAS/RNase L-dependent pathway) as well as the antiviral activity (the putative OAS/RNase L-independent pathway) against WNV infection. Moreover, several cases of WNV infection in young ostriches were reported. Therefore, current study aimed to investigate the antiviral activity of OsOAS-L against WNV infection. Both, OsOAS-L as well as ChOAS-L were ectopically expressed in BHK-21 and HeLa cell lines and investigated the antiviral activity against the WNV-replicon infection, protein localization, and enzymatic activity. The obtained results demonstrated that, both ChOAS-L and OsOAS-L proteins localized into the cytoplasm. Moreover, the OsOAS-L protein expresses the antiviral activity against WNV infection as well as the ChOAS-L, where, the cell express the OsOAS-L protein suppress the replication of WNV-Replicon as well as ChOAS-L in comparison with both shame and control infection groups. Further investigation is required to uncover the mechanism underlying the antiviral properties of avian OAS proteins against Flavivirus infection, which will contribute to control the spread of Flaviviruses.

Keywords: ostrich, oligoadenylate synthetase gene, West Nile virus.

S3- 0147 The effect of taurine on chicken small intestinal inflammation

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Chicken intestinal disease is one of the important factors which can cause damage to the poultry industry and human health. Taurine has been found to be the most abundant free amino acid in many tissues and involved in various physiological functions. Here we explored the ameliorative effect of taurine on LPS-induced small intestinal inflammation. Results showed that LPS decreased the number of proliferation cells, goblet cells and reduced secretion of MUC2 compared with controls. Real time RT-PCR also revealed a significant up-regulation in the mRNA expression of various proinflammatory cytokines (IL-1 β , IL-8 and TNF- α) and down-regulation of MUC2 mRNA and Bcl-2 mRNA expression. In addition, the production of malonaldehyde (MDA) in small intestine increased and the activity of superoxide dismutase (SOD) dramatically reduced after LPS stimulation. However, simultaneous supplementation of taurine (60mg/kg) markedly alleviated the LPS-induced inflammatory effects by down-regulating inflammation-related and apoptosis-related gene expression and restoring the antioxidative level. In summary, our findings indicated that taurine might potentially ameliorate small intestinal inflammation and provide novel insights into the underlying mechanism of anti-inflammation effect of taurine.

Keywords: taurine, LPS, chicken intestine; inflammation, mucin

S3-0148 A preliminary study on the pathogenicity of avian infectious bronchitis virus

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Infectious bronchitis virus (IBV) is an important pathogen in chickens, which might increase susceptibility to infection with other pathogens resulted in higher mortality, reduced egg production, and deteriorated egg quality. Most of these strains can cause specific lesions in the respiratory tract, but some may play part in kidney or genital tract. In order to investigate the pathogenicity of IBV, the TW-1 virus was selected for animal experiment. We randomly allocated 1-day-old SPF chickens (n = 55) into four groups. Chickens in groups 1, 2, and 3 (fifteen chickens per group) were inoculated with IBV ck/CH/LDL/140520 in 1-day-old, 15-day-old, and 30-day-old, respectively. The rest ten chickens were in group 4 as control. All birds were monitored until 120 days for the presence and severity of clinical signs indicative of IBV. The result showed that obvious respiratory symptoms were observed between 3 and 10 days post challenge in all the chickens in groups 1 and 2. The mortality rate was significantly higher in group 1 than that of group 2. Gross lesions mainly confined to the kidneys in the dead, and some lesions were found in fallopian tubes in the live. The morbidity and mortality were 0% in group 3, but some lesions were observed in fallopian tubes of 120-day-old chickens. No clinical signs were seen in the control. Therefore, the results demonstrated that chickens challenged with the same virus showed different symptoms at different days which might related to the age. Although no clinical signs were seen in some challenged ones, different degrees of damage were found in the fallopian tubes. And no IB challenge virus was isolated from the chicken challenged with IBV by the ending of the experiment.

Keywords: IBV, pathogenicity, clinical signs

S3-0149 Identification of differentially expressed microRNAs in chicken spleen with *Salmonella enteritidis* infection by RNA sequencing approach

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Salmonella enteritidis (SE) not only causes significant economic losses in poultry production, but also is of great concern to human health. The objective of this study was to identify miRNAs associated with SE infections in chickens. 3-day-old Specific-pathogen-free chickens are orally inoculated with 1.0 ml of the bacterial suspension containing 108 colony forming unit (cfu) of SE. Based on the bacteria burden and clinical symptoms, we divided the chicken into three groups, called control (C) group (0cfu & alive), susceptible (S) group (107cfu & dying) and resistance (R) groups (105cfu & alive), respectively. Spleen tissues of three individual chickens were selected for Solexa sequencing from each group. And the results of differentially-expressed (DE) were verified by using qRT-PCR. The results showed that 20 (9 up- and 11 down-regulation), 13 (9 up- and 4 down-regulation) and 12 (10 up- and 2 down-regulation) DE miRNAs, as well as 526 (370 up- and 156 down-regulation), 107 (79 up- and 28 down-regulation) and 176 (101 up- and 75 down-regulation) DE genes were identified in S vs. C, R vs. C and R vs. S, respectively. Gene ontology and KEGG pathway analysis revealed that these microRNAs could target genes enriched in the cytokine-cytokine receptor interaction and NF- κ B signaling pathway. Moreover, seventeen DE genes and ten DE miRNAs were validated via qRT-PCR. The fold changes of 15 DE genes (88%) and 8 DE miRNAs (80%) were strong consistency by using the qRT-PCR and RNA-Seq test. A comprehensive analysis combining both miRNA and targeted mRNA gene expression suggests that gga-miR-155, 130b, 30, 101 and SOCS1, CTLA4, IRF4, LRRC59 are strong candidate miRNAs or genes involved in regulating the host response to SE infection in chickens. Elucidation of the mechanism of these miRNAs on the regulation of host-SE interaction will lead to the development of new control strategies to prevent or treat SE infections in poultry.

Keywords: chicken, microRNA, *Salmonella enteritidis*, RNA sequencing, differentially expressed

S3- 0150 Occurrence and genotypes of Campylobacter in broilers during rearing period

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Campylobacter is one of the most important foodborne bacteria which cause gastroenteritis in humans worldwide. Poultry is recognized as a main source of Campylobacter infection in humans. To understand the dynamics of Campylobacter during the broilers production process and the genetic diversity of strains in broilers and environmental samples, Campylobacter isolates were recovered from broilers and environments in nine chicken flocks in two farms during their growth process. The genetic relationship of Campylobacter isolates was determined by multilocus sequence typing (MLST). Flocks were colonized as early as 3 weeks after introduction to the farm. The highest colonization rate was more than 90%, which occurred in the following 4 - 6 weeks. Quantitative data showed that the highest Campylobacter loads appeared at 1 - 2 weeks after initial colonization. Campylobacter load of the cloacal swabs in 4 flocks at 5 weeks was significantly higher than that at 3 weeks ($P < 0.05$). MLST of 171 selected Campylobacter isolates resulted in 20 sequence types (STs), which consisted of 12 STs for *C. jejuni* and 8 STs for *C. coli* isolates. The ST type of Campylobacter isolated from farm 1 was more diversified than that from farm 2. The ST type of the environmental samples was highly consistent with that of animal swab samples. This work highlighted prevalence and contamination load of Campylobacter in broilers during their rearing period. The consistency of Campylobacter STs in environmental and cloacal swab samples suggested that the environment may be one of the main sources of chicken infection.

Keywords: Campylobacter, occurrence, genotype, broiler, rearing period

S3- 0151 Efficacy of the Bacillus licheniformis probiotic OptiBac- L[®] administered in the feed for the control of Necrotic Enteritis caused by Clostridium perfringens in broiler chickens

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The objective of the 42d floorpen study was to determine the benefit of feeding the probiotic OptiBac-L[®] to broiler chickens in order to reduce *Clostridium perfringens* (CP) induced Necrotic Enteritis. OptiBac-L[®] is a direct fed microbial for broilers intended to provide live microorganisms in which the active ingredient is spray-dried spore forming bacterium *Bacillus licheniformis*, with a minimum concentration of 3.2×10^9 viable spores/gram. The treatment groups were: 1. No additive, no CP (NMNI); 2. no additive, CP (NMI); or 3. OptiBac-L[®] 2 kg/mt, fed continuously in feed. A randomized block design with 8 replications of 50 birds per pen was used. All chicks were vaccinated at hatch with a commercial coccidia vaccine. On d19, 20 and 21 all birds, except NMNI were challenged with CP (1×10^8 cfu/bird). On d21, five birds per pen were scored for NE lesions (scoring 0-3). Bird weights and feed consumption were measured on d21, 35, and 42. All weights were in kilograms and significance was set at ($p < 0.05$). This study reproduced clinical Necrotic Enteritis (NE) (9.3 % NE mortality for NMI). The adjusted feed conversions at all weigh periods were significantly improved for OptiBac-L[®] compared to NMI (d0-21 (1.529 vs 1.554), d0-35 (1.655 vs 1.729), and d0-42 (1.749 vs 1.831). Average weight gains were significantly improved for OptiBac-L[®] compared to NMI at d35 (1.589 vs 1.396) and d42 (2.192 vs 1.971). OptiBac-L[®] fed bird's d42 FCR and weight gain were statistically equivalent to the NMNI birds. NE was reduced by feeding OptiBac-L[®] with significantly lower % NE mortality (1.9 % vs NMI 9.7%) and NE lesion scores (0.58 vs NMI 0.83). In conclusion, the feeding of OptiBac-L[®] demonstrated significant improvements in performance as well as reducing Necrotic Enteritis in coccidia vaccinated broilers.

Keywords: OptiBac-L, Bacillus, Necrotic Enteritis, probiotic, Clostridium

S3-0152 The drinking water quality of poultry farm in Shandong province China

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The water quality is very important for birds health. To investigate the water quality of poultry farm in Shandong province China, 70 well water samples were collected and detected for the number of *Escherichia Coli*. Then we also chosen 3 points in one waterline in every farm to detected for the number of *Escherichia Coli*. Fifty-four of these farms had between 10000 and 30,000 bird flocks (medium-sized flocks) and 16 had more than 3,0000 birds (big-sized flocks). And sixty farms were housed in cage production systems, another farms were in cage-free production systems. The results indicated that 30 of 70 (42.9%) well water samples were qualified, 40 of 54(74.1%) well water of big-sized farms were qualified. And the water quality of cage production systems were better than cage-free ones. We detected different points of 140 waterline samples, the results demonstrated that the number of *Escherichia Coli* of middle point in one waterline was more than the end point. These findings indicated that the water quality of big-sized farm was better than the medium-sized ones, and we should take more attention to the different site in one waterline.

Keywords: water quality, *Escherichia Coli*, waterline

S3-0153 Assessment of immune effect about ND- IB- EDS inactivated vaccine in farm

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The study was designed to investigate the immune effect of ND-IB-EDS inactivated vaccine produced by company A and B on infectious bronchitis (IB). 12000 healthy CAU (China Agricultural University) No. 3 Layer in the same pheasantry were randomly divided into two groups, which were injected with A and B vaccine, respectively. The experimental period lasted for 90 d. The blood samples were collected from randomly selected 64 laying hens on 30 d, 45 d, 60 d and 90 d after immunization, and then the serum was sterily obtained. Finally, the serum antibody titers were detected by IBV antibody ELISA kit produced by IDEXX company. Average antibody titers of layers in two groups increased from 0 d to 60 d, and then gradually reduced from 60 d to 90 d, with the peak in 60 d of 6828 and 16095, respectively. Compared with vaccine A, the antibody titer of vaccine B in the same period (30 d, 45 d, 60 d and 90 d) was significantly higher ($P<0.05$) (antibody titer of vaccine A vs B: 2202 vs 5071; 2443 vs 7230; 6828 vs 16095; 1606 vs 6835), and showed a significantly lower coefficient of variation ($P<0.05$) (vaccine A vs B: 65.4 vs 49; 99.4 vs 59.7; 110.7 vs 59.1; 76.2 vs 52.9). Vaccine B could maintain higher antibody level, the larger peak of antibody titers and longer period. Hence, the immune effect of vaccine B was better than vaccine A.

Keywords: CAU-No. 3 layer; infectious bronchitis (IB); antibody titers

S3- 0154 Gizzard Erosion in broilers: A silent killer of broiler health and performance

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Broiler industry in Asia has been playing a pivotal role in Food Security in the region, which is home to nearly a half of the mankind with increasing middle class. The industry seeks to maintain growth rate, sustainability and lead in its contribution to Food Security. That requires an understanding of some of the finer aspects of chicken health and management, their impact on performance and possible causes. Gizzard Erosion (GIZ) is one such issue that was known to be caused by toxic amines, mycotoxins etc in feed. Often this condition was seen without clinical signs or mortality. An attempt has been made in this study to estimate the occurrence of GIZ and its impact on sensitive organs like intestine, proventriculus (PRV) and bursa damage (BDM) and on broiler weight (BOW). Data were obtained from Elanco's Health Tracking System (HTSi), a global poultry health surveillance system. This system involved performing standardized routine necropsies on a representative convenience sample of five birds per flock, including assessment of 23 intestinal health conditions. Data of nearly 18, 500 birds, representing 3,700 flocks of Asia during 2013 to 2015 were used for the analysis. We did the bivariate analysis and Tukey - Kramer t-Test for finding the association and its influence. By this analysis we found that there is a positive association between GIZ and excessive intestinal mucus content, BDM and PRV ($P < 0.01$). Also, birds with GIZ had lower BOW than birds without GIZ. This growth depression is more severe as the severity of GIZ increases ($P < 0.01$). In this analysis we also discovered that the occurrence of GIZ was substantially high in Asia. Considering facts that GIZ is not apparent by clinical signs, has high occurrence and negative influence on BOW, they bring upon the industry a significant economic loss. Further studies are required to understand causes and solutions; and thereby to improve the broiler production efficiency

Keywords: broiler, health, broiler weight, gizzard erosion, surveillance

S3-0158 A survey of infectious bronchitis virus in broiler breeders and broilers in Thailand

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The aim of this study was to monitor the persistence of IBV vaccines and IBV field challenge in broiler breeders and broilers in Thailand between March and September, 2015. Non related to IBV clinical appearance, Sixty randomized samples from three farms of broiler breeder chickens were taken at 2, 7, 8, 22, 28, 29, 49, 58 and 59 weeks of age. Thirty-five samples were taken from two farms of broilers at 3, 4 and 5 weeks of age. All chickens were vaccinated against IB with live vaccines according to label directions. To evaluate the prevalence of each strain of IBV, tissue samples from tracheas, lungs, kidneys and cecal tonsils were applied to FTA cards. Samples were sent to X-OVO limited, UK to identify the strain of IBV by RT-PCR, followed by S1 gene sequencing. In broiler breeders, the viruses were detected in 19 samples (31.7%). Twelve positive samples of IBV 4/91 vaccine strain were detected in 2, 3, 7, 8, 28 and 29-week-old breeders. Two positive samples of IBV 793B strain were detected in 7 and 29-week-old breeders. Three positive samples of IBV QX-like strain were detected in 22, 48 and 58-week-old breeders. One positive sample of IBV Ark strain was detected in 58-week-old breeders. In broilers, the viruses were detected in 12 samples (34.3%). Two positive samples of IBV 4/91 strain were detected in 4 and 5-week-old broilers. One positive sample of IBV D274 strain was detected in 5-week-old broilers. Nine positive samples of IBV QX-like strain were detected in 3, 4 and 5-week-old broilers. The results reveal the circulation of IBV 4/91 vaccine strain which is the same as vaccine strain in the vaccination programs. This study also confirms the circulation of IBV QX-like strain and the emergence of IBV 793B, Ark and D274 strains. These survey findings will help owners as well as attending veterinarians to acknowledge and emphasize control measures and evaluate the vaccination program accordingly.

Keywords: infectious bronchitis, RT-PCR, QX-like IBV, Thailand

S3-0166 The use of dietary supplements to reduce the incidence of fatty liver syndrome in laying hens

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The current study assessed the effect of two dietary supplements (FLS- MIX and FLS- LIQ; Trouw Nutrition) that aim to reduce the occurrence of fatty liver syndrome (FLS) in layers. The FLS is a metabolic disease that can potentially reduce egg production while compromising the health status of the bird. Nutritionists have been seeking for dietary tools to reduce the prevalence of FLS thereby enhancing laying performance and animal welfare. Dietary supplements were provided through the feed (FLS- MIX; 10 kg/t) or the drinking water (FLS-LIQ; 0.25 % v/v). A total of 288 individually caged Hy- line brown hens (60 weeks) were blocked per BW before the start of the study. The experiment followed a 2 diets (Standard; Challenge [higher starch and energy to protein ratio]) \times 3 treatments (None; FLS-MIX, FLS-LIQ) factorial arrangement of treatments. After offering the Standard or Challenge diet during 14d to induce the FLS, hens were maintained on the same diet but received their corresponding treatment. Laying performance was determined during 6 consecutive weeks. At the end of the experimental period, hens were slaughtered to evaluate the liver status. The Challenge diet successfully induced the FLS as evidenced by reductions in daily feed intake, lay percentage and a greater liver lipid content compared to the Standard diet ($P < 0.05$). No differences in lay performance or egg-shell quality were observed among the different treatments, although FLS- MIX significantly increased ($P < 0.05$) feed intake relative to None. On birds subjected to the Challenge diet, FLS-MIX and FLS- LIQ resulted in lower ($P < 0.05$) liver fat content relative to None (205, 245, 274 g/kg DM for FLS-MIX, FLS-LIQ and None, respectively). In contrast to FLS- LIQ, FLS-MIX decreased ($P < 0.05$) liver friability and the presence of necrotic spots compared to None. Results suggest the usage of dietary supplements can be an effective means to reduce the deposition of fat into the liver when hens are affected by FLS.

Keywords: fatty liver, layers, metabolic disease

S3-0167 Specific egg yolk immunoglobulin (IgY) against experimental *Vibrio harveyi* infection in the Japanese pufferfish (*Takifugu rubripes*)

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Vibrio harveyi has been recognized as a primary pathogen that causes the skin ulcer disease of Japanese pufferfish in recent years, which results in high mortality and enormous economic losses to aquaculture. Currently, the common method to control *V.harveyi* is by administration of antibiotics. However, excessive or misuse of antibiotics can lead to bacterial resistance. Therefore, it is necessary to find an alternative strategy. The aim of this study was to prepare specific egg yolk immunoglobulin (IgY) against *V.harveyi* and evaluate its protective effects. Specific IgY was produced by Hy- Line variety brown immunized with formaldehyde-killed *V.harveyi*. The purified IgY powder was prepared from egg yolk with the method of water dilution, salting out, ultrafiltration concentration and vacuum freeze drying. The passive immunization effects of egg yolk powder and IgY powder against *V.harveyi* were evaluated in pufferfish. In oral administration experiment, the fishes fed a diet containing 1%, 5% and 10% specific egg yolk powder for 7 days and then intramuscular injection of *V.harveyi* showed survival rates of 20.0%, 30.0% and 50.0% at day 10, respectively. However, the fishes treated with 10% non-specific egg yolk powder only gave survival rate of 13.3%, while no treatment fishes all died. In immersion administration experiment, immersing fishes into rearing water containing specific IgY powder provided significant protection effect against *V.harveyi* infection ($P < 0.05$). The survival rate of 300 mg/L pre-immersion group was 63.3% and the non-specific IgY group was 6.3%. In conclusion, the study suggested that specific IgY has potential to control *V.harveyi* infection in the Japanese pufferfish.

Keywords: egg yolk immunoglobulin, *Vibrio harveyi*, Japanese pufferfish, skin ulcer disease, passive immunization

S3- 0168 Study on the chondrocyte apoptosis levels in Tibial Dyschondroplasia broilers induced by thiram

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Tibial Dyschondroplasia (TD) is a kind of bone disease happened along with the rapid growth of broiler. In the previous study, we successfully screened out the differentially expressed genes (DEGs) related to apoptosis in chondrocytes TD broiler by gene chip. This study was undertaken to further examine the apoptosis level of chondrocytes from different cartilage areas in TD broiler. To this end, HE staining and TUNNEL technology was respectively used to detect the structural damage and apoptosis of chondrocytes. Results showed that at 1, 2, 4 and 6 day after thiram exposure, lesion zones of growth plate increased and chondrocyte structural damage in broiler tibia aggravated gradually which was characterized by the reduced cytoplasm, increased empty cartilage capsule and damaged cells in proliferation zone. Apoptosis levels of the chondrocytes in cartilage lesion parts especially in the hypertrophic zone significantly increased. For revealing the possible reasons of chondrocyte apoptosis in TD broiler, gene expression changes of genes involving in cell apoptosis were analyzed by real-time PCR. As the results shows, mRNA transcription level of Bax was significantly up-regulated at all test time from day 1 to 6 ($P < 0.01$). Consistent with the Bax changes, caspase-3 expression also significantly increased at 1, 2 and 4 day ($P < 0.01$) in TD broiler chondrocytes. Conversely, there was a significant reduction of BAG1 gene expression at 1 and 2 day after thiram treatment, which indicates that anti-apoptotic genes could regulate chondrocyte apoptosis during cartilage growth of TD broiler. In conclusion, the occurrence of TD associated with chondrocyte apoptosis in lesion cartilage and further investigations on the interaction mechanism are warranted.

Keywords: Tibial Dyschondroplasia, thiram, apoptosis, chondrocyte

S3-0169 Immune responses induced by different live infectious bronchitis virus vaccination regimes and the protection conferred against infectious bronchitis virus Q1 strain

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The objective of this study was to assess the mucosal, cellular and humoral immune responses induced by two different infectious bronchitis virus (IBV) vaccination regimes and their efficacy against challenge by a variant IBV Q1. Day-old broiler chicks were vaccinated with live H120 (Massachusetts serotype) alone (Group I) or in combination with CR88 (793B serotype) (Group II). Both groups were again vaccinated with CR88 at 14 days of age (doa). One group was kept as a control (Group III). All groups were challenged oculonasally with a virulent Q1 strain at 28 doa, and their protection was assessed. Chicks vaccinated with H120 and CR88 at day-old, followed by CR88 at 14 doa (Group II), showed significantly higher CD8+ responses in the trachea and higher lachrymal IgA levels compared to those vaccinated with H120 alone (Group I). In terms of ciliary protection against Q1, though both vaccinated groups were protected, the combined vaccination of H120 and CR88 of day-old chicks, followed by CR88 at 14 doa, showed higher ciliary protection and less RNA load in trachea and kidneys, and histopathological lesions were reduced. This study highlights the modulation of chick immune responses with the use of currently available Massachusetts and 793B live vaccines, so that better protection against field variant IBVs can be afforded.

Keywords: IBV, vaccination programmes, immune responses, protection against Q1

S3- 0170 Identification of immune-related gene expression in chicken red blood cells

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Traditionally, red blood cells are well known by people for their essential role in cell respiration by transporting of O₂ and CO₂. In recent years, it has been found that red blood cells are associated with the body's immune response and regulation. In our previous study, we successfully screened a large number of differentially expressed genes (DEGs) related to immunity in red blood cells infected by Marek's Disease Virus (MDV) through transcriptome sequencing. In the present study, we aim to identify the expression of these DEGs in red blood cells by RT-PCR, which is an indispensable step for their biological function study. The total RNA of chicken red blood cells was extracted and all the DEGs were amplified by RT-PCR through designing corresponding primers. Lengths of the PCR products were verified by DNA agarose gel electrophoresis. As the result shows, 8 DEGs were successfully identified expression in chicken red blood cells which include TLR 4, TLR 7, TRAF 6, TRAF 3, MYD 88, MHC II, MHC I and IFN- β . A clear single band was observed on each target gene which could be used in further studies.

Keywords: Marek's disease virus, red blood cell, transcriptome sequencing, immunity

S3- 0176 Allicin alleviates immunosuppression induced by reticuloendotheliosis virus infection in SPF chickens

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In the present study, we aimed to determine the therapeutic effect of allicin on immunosuppression induced by Reticuloendotheliosis virus (REV) infection in SPF chickens and discuss the mechanisms of action. One day old SPF chickens were randomly divided into control group (n=8), model group (n=8), allicin treated group (300mg/kg, n=8) and allicin treated separately group (n=8). Immunosuppression chicken models were constructed by 100 tissue culture infective dose (TCID₅₀) REV intraperitoneal injection while the control group was inoculated with normal saline. Spleen, thymus and bursa of fabricius were sampled at day 35 post infection. The levels of mRNA of REV related inflammatory factors were measured by realtime fluorescence quantitative polymerase chain reaction (RT-PCR) and protein expressions of related inflammatory signaling pathways were checked by western blotting. As a result, the REV infected chickens revealed high expression of inflammatory factors such as IL-6, IL-1 β , IFN- γ , TNF- α and IgA along with atrophy of thymus as well as bursa of fabricius and swelling of spleen, while allicin could markedly alleviate inflammatory reaction in immune organs. Moreover, allicin significantly inhibited inflammatory signaling pathways such as nuclear factor κ B (NF- κ B) and mitogen-activated protein kinase (MAPKs) including JNK1/2, ERK1/2 and p38 phosphorylation induced by REV, indicating a post transcriptional mechanism of this modulation. In conclusion, allicin is potential to be used for virus treatment as an anti-inflammatory nutraceutical.

Keywords: allicin, reticuloendotheliosis virus, NF- κ B, MAPK, SPF chicken

S3- 0177 Effect of Farm- O- San AHS product on performance and blood stress parameters of animals under heat stress

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The objective of this study was to measure the effect of the supplement Farm-O-San AHS on performance (body weight, weight gain, feed intake, feed conversion rate and mortality) of animals under heat stress conditions (35° C during 4 hours per day). The study was executed at the Trouw Nutrition R&D Poultry research centre. The trial consisted of 4 treatments, which varied in the type and level of product added to drinking water: no product, 1 kg/1000 L Farm-O-San AHS, 2 kg/1000 L Farm-O-San AHS or 200 g/1000 L Farm-O-San Vitamin C. One thousand twenty four male Ross 308 broiler chickens were used. Performance (body weight, feed intake, mortality) was monitored from 0 to 36 days of life. Heat stress (35°) was applied during 4 hours/d throughout the entire experimental period. The remainder of the day animals were held under standard temperature. Animals which consumed the highest level of Farm-O-San AHS or Vitamin C showed higher weight gain, or feed intake ($P < 0.01$) than the control animals. The lowest feed conversion rate was found in animals drinking the highest level of Farm-O-San AHS ($P = 0.03$). Farm-O-San AHS has a positive effect on broiler chickens performance under heat stress conditions, improving animal's growth and feed conversion.

Keywords: chickens, heat stress, drinking water, supplement, mortality, performance

S3- 0178 Biological characterization of clade 2.3.4.4 H5NX subtype avian influenza viruses

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The highly pathogenic avian influenza H5 viruses with various NA subtypes (H5NX) are reported varying along with the evolution of H5N1 viruses by reassortment and mutation, evaluating their risk of damage to both poultry industry and public health is of great importance. Here, one H5N1 and three reassortants of H5N2, H5N6 and H5N8 subtypes of the current prevalent clade 2.3.4.4, isolated from Eastern China in 2014, were characterized biologically. In vitro viral growth curves indicated that all the three reassortants replicated to high titers in CEF, DEF, MDCK and A549 cells as the H5N1 virus, but to significantly lower titers in Vero cells. Pathogenicity studies in vivo showed that all these viruses were highly virulent to chickens and mallard ducks, while they showed moderate to high virulence in mice. Additionally, the solid-phase direct binding assays with glycans demonstrated that the H5N1, H5N6 and H5N8 viruses could bind to both avian type SA α -2,3Gal and human type SA α -2,6Gal receptors, but the H5N2 reassortant just preferred the former. Our findings clarified that the clade 2.3.4.4 H5NX subtype reassortants were tremendous harmful to poultry industry and with potential threat to public health, highlighting the necessity of taking effective prevention and control measures.

Keywords: H5NX avian influenza virus, clade 2.3.4.4, virulence, receptor

S3-0179 Development of a duck hepatitis A virus vaccine

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Duck viral hepatitis (DVH) is an acute and fatal disease of young ducklings characterized by liver enlargement and hemorrhage by postmortem examination. In China, DVH is a common disease because the occurrence of DHAV-3 around 1999. In this study, we describe the development of a DHAV-3 vaccine candidate which can provide effective protection against the virulent DHAV-3 challenge in the ducklings. DHAV-3 HB strain was isolated from the liver sample that was collected from dead ducklings with typical DVH lesions. To develop vaccine, the strain was serially passaged on 8- to 9-day-old embryonated-pathogen-free chicken eggs and the 80th passage did not cause illness in ducklings, suggesting its pathogenicity was attenuated. To test the immunogenicity, one group of 10 1-day-old ducklings were subcutaneously inoculated with 103.5 ELD₅₀ of the DHAV-3 HB80. Another group were served as control. Both groups of ducklings were challenged with virulent DHAV-3 on day 7 post-inoculation. Result showed that immunization with HB80 provided 80% protection in ducklings from the virulent DHAV-3 challenge, while the mortality in the non-immunized control group was 80%, with typical clinical signs and gross lesions of DVH. To evaluate the safety, the virulence reversion test of the strain HB80 was conducted by five continuous back-passages in 2- to 6-day-old ducklings. No duckling developed any clinical symptom during the trial and the virus was not detected from the duckling liver tissue from the third back-passage. No gross or microscopic lesion was observed in ducklings of all passages. Biochemical test of the blood samples collected from immunized ducklings did not show significant difference from the non-immunized ducklings. In conclusion, our results demonstrated that the live attenuated virus DHAV-3 HB80 was safe and could provide effective protection from the virulent virus challenge in immunized ducklings. More studies will be done in the future.

Keywords: DHAV-3, HB80, virulence reversion, immunogenicity

S3-0180 A study on the prevalence of microbial disease in broiler flocks of Kamrup (metro) and Kamrup (rural) districts of Assam

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A study was conducted in order to find out the common microbial diseases of the broiler farms in two districts of Assam namely, Kamrup (Metro) and Kamrup (Rural) district. The data were collected from 100 nos of farms selected randomly during the period from end November, 2013 to mid December, 2014. The clinical signs of the diseased flocks were recorded, farmers were interviewed for any other symptoms they noticed and in case of any mortality during the personal visit post mortem examination was performed to observe gross pathological changes. From the affected flock blood samples were collected for microbiological study. The study showed that broiler farms during the period of study encountered various microbial diseases where highest incidence recorded was Hydropericardium syndrome (HPS) (16.75%) followed by Omphalitis (13.40%), Colibacillosis (11.11%), Chronic Respiratory Disease (CRD) (10.93%), Infectious Bursal Disease (IBD) (10.58%), Bronchitis (9.88%), Necrotic Enteritis (6.35%), Bacillary White Diarrhoea (5.11%), Newcastle Disease (4.59%), Brooder Pneumonia (3.70%), Gangrenous Dermatitis (2.82%), Laryngotracheitis (2.12%), Coryza (1.59%) and Bumble foot (1.06%). Kamrup district encountered comparatively higher disease incidences than Kamrup (Metro) district.

Keywords: broiler, microbial disease

S3-0182 S2 subunit of spike protein (S) of avian infectious bronchitis virus plays a critical role in the cellular infect progress

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The avian coronavirus, infectious bronchitis virus (IBV), is an important poultry pathogen. Most of IBV have strict cell tropism, except a lab adapted strain, Beaudette, which can grow on vero cells. Such properties cause a lot of limitations to the study of the virus on the cell level. Thus, it becomes important to study the molecular mechanism of IBV in cell tropism. This study is based on the IBV reverse genetic system constructed by our team, cross to replace S gene of vaccine strain H120, the CK cell adapted strain and kidney type virulent strain IBYZ, which is CK cell non-adapted strain, and successfully rescued the recombinant virus rH120-S/YZ, rIBYZ-S/H120, and their parental clones rH120, rIBYZ. After infected CK cells, the relationship between S protein and cell tropism of IBV was studied from the aspects of cell pathological changes, immunofluorescence and virus copy number. As a result, we found that rH120-S/YZ, the S gene of which replace by IBYZ, lost the original CK cells' tropism, while rIBYZ-S/H120 obtained the capable of CK cells' infection, which demonstrated that protein S was a decisive factor of IBV in cell tropism. In the process of virus invasion, the S protein is cleaved into two subunits of S1 and S2, and the functional domains of S1 have receptor binding properties, and the S2 function domain is related to the fusion mechanism. In order to further explore the correlation between the two subunits and the CK cell tropism, we take IBV H120 as a skeleton, construct the recombinant virus, which chimeric expressed S1 or S2 gene of the ck cells non-adapt strains IBYZ or M41. After the cell lesion was observed, rH120-S1/YZ and rH120-S1/M41, which remained the S2 gene of H120, could still cause the cytopathy, while rH120-S2/YZ and rH120-S2/M41 lost the capability of ck cells' infection. Therefore, we suppose that the S2 gene plays a critical role in the cellular infect progress of IBV, but the key sites still need to be further studied.

Keywords: infectious bronchitis virus, spike protein, cell tropism, reverse genetics

S3- 0183 Coccidiosis: updates on a Brazilian perspective (2013 – 2015)

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Coccidiosis is one of the most important causes of economic losses within the poultry industry. Little is known about the prevalence of coccidiosis in Brazil. Our objective in this study was estimate the prevalence and average score of coccidia injury in the different Brazilian regions (South, Southeast and Midwest) from 2013 to 2015. The Brazilian production of chicken meat reached 13,146 million metric tons in 2015. This consolidated Brazil as the second largest producer in the world and the leader in the world ranking of exports. However, due to the Brazil's large territory, productivity and health challenges are different in its various regions. Coccidiosis is caused by protozoa of the genus *Eimeria* and main species are *E. acervulina* (gAc), *E. maxima* (gMx) and *E. tenella* (gTn), which have varying degrees of pathogenicity and zones of infection of the intestinal tract. Although high mortality outbreaks are not common, coccidiosis can dramatically affect the performance and cause significant intestinal disorders. Elanco's Health Tracking System (HTSi[®]), a global poultry health monitoring program over time, was used to obtain the data recorded from standardized necropsies. Approximately 73,960 birds, representing 13,655 Brazilian flocks between 2013 and 2015 were investigated. The results showed that lesions by *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella* were respectively 14.26% , 6.58% , 4.81% in South Region; 12.41%, 6.81%, 7.80% in Southeast and 2.17%, 5.25%, 1.43% in Midwest. The average score of gAc (0.14) was higher in South and the average score of gMx (0.08) and gTn (0.08) was superior in Southeast. This study maps the behavior of coccidiosis in Brazil during of period analyzed. Considering the economic loss and health impact of coccidiosis, the relevance of this data is to help chicken producers and veterinarians to understand the coccidiosis challenges in each Brazilian region and to take correct actions about this illness.

Keywords: chicken, coccidiosis, intestinal health, monitoring

S3-0184 Implantation of ileal and cecal microbiota in *Anas platyrhynchos* (Pekin), *Cairina moschata* (Muscovy) and mule ducklings using Miseq sequencing

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Intestinal microbiota plays a major role in physiology and forms a complex ecosystem. For example, intestinal bacteria can affect gut morphology, nutrition and is known to stimulate immune response and to protect against pathogens. Previous works in the lab allow us to identify Firmicutes and Bacteroidia as the dominant phyla in ducks. Overfeeding increased the relative abundance of Lactobacillaceae family in the ileum. To access the implantation of microbiota in Pekin, Muscovy and mule ducklings, the ileum and ceca digestive contents of five animal by genetic type and days of growth (Day 2, 3, 5, 12, 15, 30, 36, 50 and 65) were studied. These analyses were performed by using miseq sequencing of the gene coding for the 16S rRNA. Whatever the digestive content, the genotype, and the growth period, Firmicutes, Proteobacteria and Bacteroides are the most dominant phyla. A sequential implantation is detected in both ileal and cecal digestive contents according to age and the type of food at the phyla level. In ceca, The ChaoI, The ACE and the Shannon index increased with age independently of genetic type. In the ileum, regarding the Shannon index, there was an interaction between age and genetic type and mostly increased with age too. Finally, a genetic effect was detected regarding Firmicutes phylum only in ileum.

Keywords: duck, Implantation of intestinal microbiota, Mi-seq sequencing

S3-0185 Construction and characterization of a full-length cDNA infectious clone of a nephropathogenic infectious bronchitis virus strain

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Avian infectious bronchitis virus (IBV) is the causal agent of the infectious bronchitis (IB), an acute and highly contagious disease of chickens. There are numerous IBV strains existed, causing pathological changes in different organs varying from respiratory tract to kidney. The nephropathogenic IBV strain ck/CH/IBYZ/2011 (GenBank No. KF663561) was isolated and sequenced in our previous study. It belongs to the Chinese QX-like genotype which initially emerged in China and caused great economic loss to poultry industry in Asia, Russia and Europe. We choose to use virulent strain of IBV as the cDNA backbone because we hope to elucidate mechanisms of pathogenesis. The full-length cDNA of IBYZ was constructed from ten cDNA fragments by using an in vitro assembly method. Capped RNA transcript from the cDNA was transcribed with T7 polymerase using the in vitro Transcription T7 Kit, and transfected into BHK-21 cells together with the transcript of nucleocapsid protein gene. At 48h post transfection, the transfected BHK-21 cells were harvested and inoculated into 11-days old SPF embryonated chicken eggs. Potential recombinant virus was isolated from the allantoic fluid which was collected at 48h post-inoculation and characterized by RT-PCR and sequencing. The rescued viruses were designated rIBYZ (GenBank No. KT750258). The biological characteristics of rIBYZ such as EID50, growth curve and pathogenicity, were tested and all of them were similar to its parent strain ck/CH/IBYZ/2011. Clinical presentation in the rescued virus group started with respiratory depression and distress, including nasal discharge, sneezing, coughing, and rales. Within 4 d after the appearance of the death, the lethality increased to 50 % of the total flocks at the 12d. Post-mortem examination of dead birds revealed dehydrated carcasses and swollen and pale kidneys with urates in the tubules. The full-length cDNA clone of rIBYZ is a valuable tool for elucidating mechanisms of pathogenesis of IBV.

Keywords: infectious bronchitis virus, nephropathogenic, reverse genetics

S3-0186 Construction and identification of recombinant infectious bronchitis virus expressing the heterologous genes

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Avian infectious bronchitis virus (IBV) is an enveloped, positive-stranded RNA virus considered to be promising vector for vaccine development. To investigate the feasibility of IBV as a vector for expressing heterologous genes, we used vaccine strain H120 as a vector to express the chicken interferon- γ (IFN- γ) gene, interleukin 2 (IL2) gene, the enhanced green fluorescent protein (EGFP) gene, eEGFP (codon-optimized EGFP) gene, phospholipase C from *Clostridium perfringens* (PLC1) gene, Haemagglutinin-Neuraminidase (HN) and fusion (F) protein gene of Newcastle Disease Virus (NDV), three S1 protein genes of IBV isolated strains. Heterologous genes were amplified by RT-PCR and inserted into the upstream of gene 5a of the IBV genome by reverse genetic techniques. Rescued recombinant viruses (H120- cIFN γ /5a, H120- cIL2/5a, H120- EGFP/5a, H120- eEGFP/5a, H120- PLC1/5a, H120- F/5a, H120- HN/5a, H120- TZS1/5a, H120- YZS1/5a, H120- WFS1/5a) were identified by RT-PCR and sequencing. The biological characteristics of rescued recombinant viruses were studied. The results showed that recombinant viruses can still lead to chicken embryo dwarf. However, compared with the wild-type strain H120, the viral titer and replication rate of recombinant virus were reduced. The heterologous genes' stabilities of recombinant viruses were analysed by serial passage on 11-days old SPF embryonated chicken eggs. Recombinant IBV stability varied depending on the gene inserted. The YZS1, WFS1 and TZS1 genes were much more stable than other heterologous genes. Codon-optimization of the EGFP gene, resulted in an increase in genome stability. The results demonstrated the stability of recombinant viruses depends on the properties of the foreign gene itself.
Keywords: infectious bronchitis virus, reverse genetics, vector, heterologous gene

S3- 0187 The role of the microbial monitoring in the prevention of poultry bacterial infections

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Resistance to antibiotics is a problem for most countries of the world. The infections are caused by resistant strains of *Salmonella*, *E.coli* and *Campylobacter* cause serious diseases and can be lethal for animals and humans. The aim of research was to monitor microorganisms circulating in poultry farms of Ukraine, sensitivity to antimicrobial drugs and to develop system of rotation for prevention of poultry's bacteriosis. It was done in lab of Sumy National Agrarian University and Ukraine's poultry farms. Microbiological monitoring was carried out with using R-biopharm's test systems. The sensitivity of isolated pathogens to antimicrobial agents was tested by serial dilutions. We found that respiratory syndrome is caused by *S. aureus*, *S. pneumoniae*, *C. perfringens*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *P. vulgaris*, *S. enteritidis*, *M. gallisepticum*, *P. multocida*, *A. fumigatus*. The intestinal syndrome is caused by *S. aureus*, *S. faecalis*, *C. fetus*, *C. jejuni*, *C. perfringens*, *E. agglomerans*, *E. coli*, *P. aeruginosa*, *P. vulgaris*, *S. enteritidis*, *S. pullorum-gallinarum*, *Y. enterocolitica*. *E. coli* were represented as O2; O4; O8; O78; O157. *Salmonellas* were identified as: *S. enteritidis*, *S. typhimurium*, *S. pullorum*, *S. gallinarum*, *S. virchow*, *S. infantis*, *S. arizona*, *S. jawa*, *S. montevideo*, *S. copengagen*. Bactericidal activity to the isolated cultures had apramycin, enrofloxacin, colistin, polymyxin, trimethoprim, tylosin, tiamulin, sulfadiazine. *P. aeruginosa* was highly sensitive to apramycin, tylosin and polymyxin. *S. aureus*, *S. pullorum*, *C. jejuni*, *E. coli* O2 were sensitive according to quinolones and cephalosporins. According the results we developed antimicrobial schemes for rotation, control and prevention poultry's bacterial diseases.

Keywords: microbiological monitoring, prevention, rotation

S3-0188 Proper vaccine application is crucial to induce and optimize the protection expected from a vaccine/vaccination schedule

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Modern techniques have given the ability to develop safe and efficacious vaccines for disease control. The convenience program approach is a total program for performance improvement, it start with field situation analysis following the diagnostic pathway, from the analysis we create an action/improvement plan and we finish with evaluation of the action plan. one of the important parts of the action plan is the vaccination program. The vaccination schedule is an investment to prevent disease and safeguard the performance; from a solid investment you have more chance on a good return. To optimize the final result of vaccination plan a number of steps have to be implemented around vaccination such as: choice of the vaccine -planning/ timing (vaccination schedule), storage and preparation of vaccine, preparation of equipment, skilled operators and correct application. The trend is that farms, flocks, hatcheries produce larger numbers of chickens and this requires the implementation of standardized procedures to avoid mistakes and make it easier to monitor. Even the best vaccine can fail because of mistakes in the vaccination procedure. In modern poultry farming we invest a lot in health programs to improve the performance. For Broilers with short life cycles the ideal would be to do as much as possible in the hatchery; how can we create optimum conditions to produce day old chicks that will go in the field with broad protection against the most threatening diseases? Immunity in Broilers is based on general chick quality (genetics, Parent stock health, feed), maternal immunity(MDA) from the parents for a diseases like ND, IB, IBD, Reo, CAV, AE and additional vaccination for active immunity. In this presentation we will discuss options to optimize vaccine application and monitoring around hatchery vaccination

Keywords: vaccine, vaccine application, efficacy, performance

S3-0189 Double recombinant HVT-based vaccines for simultaneous protection against Newcastle disease, Marek's disease and infectious laryngotracheitis

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Infectious laryngotracheitis virus (ILT), Newcastle disease virus (NDV) and Marek's disease virus (MDV) are three of the most important pathogens afflicting the poultry industry and infections with these viruses result in substantial economic losses worldwide. First generation recombinant HVT-based vaccines such as HVT-ND and HVT-ILT have proven their utility in the control of diseases and production losses caused by these pathogens. However, attempts to simultaneously vaccinate with these monovalent HVT based vaccines indicated that they may interfere with each other causing delays in protection against one of the pathogens. In order to provide simultaneous protection against NDV, ILT and MDV from one viral vector, we have constructed double recombinant HVT vaccines that concurrently express the F protein from NDV and the gD and gI proteins from ILTV. Recombinant viruses were produced by plasmid based methods and one of the double recombinant vaccine viruses identified following plaque purification was characterized for stability in cell culture and other parameters related to animal safety and environmental impact. Vaccination/challenge experiments were also carried out to further assess the protection afforded by this recombinant virus by challenging birds with virulent ND, ILT or Marek's viruses and the extent of protection was evaluated following both in ovo and subcutaneous routes of administration. The results of this work are described.

Keywords: HVT recombinant Newcastle infectious laryngotracheitis

S3-0190 Sphereon®: a novel vaccine formulation technology for the storage and preparation of live poultry vaccines: the case of the Nobilis® IB Primo Qx vaccine

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Traditionally live poultry vaccines require freeze drying in glass vials and storage under vacuum in order to be stabilized for long term storage. Preparation of such vaccines for use requires a considerable number of steps prior to vaccination, making it inconvenient especially when vaccinating a large number of birds. Furthermore the volume of the glass vials limits the number of doses that can be stored in a single vial. We have developed a novel technology, Sphereon®, that gives advantages in the storage (in a recyclable compactable aluminium cup) and reconstitution of vaccines, provides improved solubility during reconstitution and allows higher dose presentations of live poultry vaccines. In addition the dose of the vaccine is adjusted according to the number of Sphereon® added to the cup, in principle, bringing more accurate dosing of the birds. The Qx strain of infectious bronchitis virus (IBV) is currently causing considerable issues in the field. Considered to have originated in China, this strain can cause respiratory disease, kidney problems and drops in egg production plus egg quality and is known to induce false layers. The development of a novel live vaccine for protection against the Qx strain (Nobilis® IB Primo Qx), safe for delivery from day old onwards, compatible with other poultry vaccines and developed with Sphereon® technology will be described.

Keywords: Sphereon, infectious bronchitis, Qx

S3-0191 Marek's disease: focus on the practical aspects of vaccination

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Vaccine failures due to the use of an insufficient dose of vaccine and/or early exposure to field virus are the most common causes of outbreaks of Marek's disease (MD). The quality of vaccine ampoules and reconstituted MD vaccine at the hatchery may be assessed by counting the number of plaque forming units (pfu) per chicken dose, which should be ≥ 1000 . An insufficient vaccine dose may also be the result of missed birds during vaccination. In any case, analysis of vaccine-take at flock level may be performed by determining the number of viraemic birds at approx. 4-7 days of age or the number of birds shedding vaccine virus via feather follicles between 19 and 21 days of age. In case of a good vaccine-take the majority of birds should show viraemia or shedding of vaccine virus via epithelial cells. Besides insufficient pfu per chicken dose, contamination of MD vaccines with Aujeszky's disease virus, avian leucosis virus, egg drop syndrome 76 virus, reovirus and reticuloendotheliosis virus has been described. Contamination of MD vaccine suspensions with *Enterococcus faecalis* at the hatchery resulting in iatrogenic-induced amyloid arthropathy and contamination of vaccination equipment with *Pseudomonas aeruginosa* causing increased mortality, have been documented also. Another important cause for vaccine breaks is early exposure, which can for instance be assessed by examination of dust samples collected at rearing farms using a differentiating real-time PCR to distinguish field virus from vaccine virus. The presence of large amounts of MD field virus in dust samples of cleaned and disinfected poultry houses forms a high risk for early exposure. Other less frequent causes of disease outbreaks are the occurrence of very virulent +/- MD field virus and increased susceptibility of birds (immunosuppression, genetic background). Both should be ruled out if evidence for a deficient vaccine-take and/or early exposure to field virus to explain MD outbreaks is lacking.

Keywords: Marek's disease, vaccination, practical aspects, early exposure, insufficient dose

S3- 0192 Granuloma disease in flocks of productive layers caused by *Tetratrichomonas gallinarum*

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In 2013, seven disease outbreaks occurred in Dutch flocks of productive layers housed on different farms. These outbreaks were characterized by increased mortality and high incidence of granulomas, mainly in ceca (340/408 hens = 83%) and livers (69/408 hens = 17%). Mortality started to increase between 21 and 35 weeks of age and reached 9.2% to 12.4% in periods ranging from nine to 48 weeks. Some flocks also showed decreased egg production and/or loss of mean egg weight. All affected flocks were linked to one rearing farm, which therefore seemed to be the source of the disease. However, no signs of disease had been observed at this rearing farm. Sentinel hens placed in one of the affected flocks to determine whether the disease had an infectious nature developed granulomas identical to those seen in the outbreaks. Next, by fulfilling Koch's postulates it was shown that *Tetratrichomonas gallinarum* was the etiological agent of the granuloma disease. The condition was reproduced in mature SPF White Leghorn hens by inoculation via both, an artificial and a natural route with a well-defined axenic *T. gallinarum* isolate obtained from one of the affected flocks. Other causes of granuloma disease were excluded.

Keywords: granuloma, layers, *Tetratrichomonas gallinarum*, ceca, liver, high incidence, increased mortality

S3-0193 Effects of dietary zinc nutrition on the intestinal barrier function and the related mechanism of broilers challenged by *Salmonella enterica* serovar Typhimurium

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The study was carried out to investigate the potential benefits and the related mechanism of zinc on the intestinal mucosal barrier function in *Salmonella enterica* serovar Typhimurium-challenged broiler chickens in a 21-day experiment. 600, 1-day-old female Arbor Acres broiler chicks were allocated into 6 groups fed with 0, 100 mg/kg of Zn in the form of either MHA-Zn or Zn-SO₄, pathogen challenge (with or without *Salmonella* challenge), and their interactions. *Salmonella* infection caused a decrease in body-weight gain and feed intake ($P < 0.05$), produced intestinal injury, as determined by a progressive increase in FITC-dextran and D-xylose in the plasma ($P < 0.05$) and a decrease in the mRNA expression of tight junctional protein ZO-1 in the ileum ($P < 0.001$). Moreover, *Salmonella* infection caused intestinal inflammation, as showed by up-regulating the mRNA expression of the inflammatory cytokines IL-8, and TNF- α in the ileum. Both organic and inorganic zinc supplementation improved growth performance by increasing the body-weight gain ($P < 0.05$) and repaired intestinal function by decreasing D-xylose in the serum ($P < 0.01$). In addition, inorganic zinc supplementation enhanced the mRNA expression of ZO-1 ($P < 0.05$) and receptor ZnR/GPR39 ($P < 0.05$) in the ileum, improved intestinal permeability by decreasing FITC-dextran in the serum ($P < 0.05$). Organic zinc supplementation alleviated intestinal inflammation by decreasing the pro-inflammatory cytokine TNF- α mRNA ($P < 0.001$) and increasing anti-inflammatory cytokine IL-10 mRNA ($P < 0.01$) in the ileum. In conclusion, zinc prevented disruption of intestinal integrity by modulating expression of cytokine gene and tight junctional protein. The major intracellular signaling mechanism may be mediated by the Zn²⁺ sensing receptor ZnR/GPR39.

Keywords: zinc, broilers, *Salmonella*, intestinal barrier, inflammation

S3- 0194 A novel high throughput chip based immuno-mass spectrometry strategy for detection and quantification of avian leukosis virus P27 and gp85

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Enzyme-linked immunosorbent assay (ELISA) and Radioimmunoassay (RIA) are the established methods to detect and quantify protein biomarkers in biofluids for various clinical applications. However, the cost of developing these assay is high since they typically require two antibodies in a sandwich format to capture the antigen of interest. Additionally, the specificity of these assay is largely affected by the quality of detection antibody and some close related antigens can not be accurately differentiated by these methods. Therefore, there is a demand for alternate assay formats to provide accurate and precise diagnostic results. Mass spectrometry analysis by far is the most sensitive and accurate method for protein identification. To demonstrate the capability of chip based immune-mass spectrometry in addressing this issue, we developed a proof of concept QLHTS assay for detection and quantification of avian leukosis virus p27 and gp85 proteins in clinical samples. The method provides a high signature peptide sensitivity through antigen enrichment and high reproducibility and robustness through a fully automated sample preparation workflow for high-throughput detection of the signature viral peptides using surface based imaging mass spectrometry. These data suggested that the surface mass spectrometry imaging based viral signature peptides analysis open numerous potential applications in the fields of proteomics and veterinary diagnostics.

Keywords: avian leukosis virus, p27, gp85, chip based immune-mass spectrometry, signature peptides

S3- 0195 Molecular cloning, expression, and functional analysis of TBK1 gene in Gallus gallus domesticus

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TRAF family member-associated NF- κ B activator (TANK)-binding kinase1 (TBK1) is a serine-threonine kinase at the crossroads of multiple interferon (IFN)-inducing signaling pathways in innate immunity. The importance of TBK1 in antiviral immunity is well established in mammal models, but in chicken, the molecular characterization and potential function of TBK1 remain unclear. So, in this study, we cloned the open-reading frame (ORF) of chicken TBK1 (chTBK1) and explore its role in anti-virus innate immune combined with small interference RNA technology. The sequencing results revealed that the chTBK1 ORF consists of 2190 base pairs (bp) encoding a deduced protein of 729 amino acid residues. Multiple sequence alignment analysis demonstrated chTBK1 similarity to other birds and mammals, which indicates that it is evolutionarily conserved. Quantitative real-time PCR (qRT-PCR) results showed that chTBK1 was ubiquitously expressed in chicken tissues and expression was especially high in immune tissues. In addition, the expression of chTBK1 was significantly up-regulated by infection with avian leukosis virus subgroup J (ALV-J) both in vivo and in chicken embryo fibroblasts (CEFs) challenged with ALV-J or stimulated with poly I:C in vitro. Consistent with the activation of chTBK1, the interferon regulatory factor 3 (IRF3) and IFN β gene in CEFs were also up-regulated after challenge with ALV-J or polyI:C. In contrast, the expression of IRF3 and IFN β in CEFs was significantly reduced by siRNA targeting the chTBK1 gene compared with a negative control (NC) during ALV-J infection or polyI:C transfection. In conclusion, our results demonstrated that chTBK1 may be an important immunoregulator for IRF3 and IFN β induction in response to viral stimulation in chicken.

Keywords: chicken, TANK- b inding kinase1, clone, function, innate immune

S3- 0196 Molecular epidemiology of infectious agents involved in respiratory disease complex in broilers in Haryana, India

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Respiratory diseases in chickens are economically important group of diseases, which may account for more than 60% cause of mortality in broilers. Most of the time there is interaction of more than one microbe in respiratory disease complex. In this study, tissue samples (trachea, lungs and air sacs) from 53 commercial broiler chicken flocks that apparently suffered from respiratory disease were tested for presence of IBV and NDV by using reverse transcription PCR and *Mycoplasma gallisepticum* and *E. coli* by using PCR. The reverse transcription PCR and PCR results showed that 41.5% and 18.8 % of these flocks were infected with NDV and IBV, respectively, whereas 18.8, 79.2% of these flocks were infected with MG and *E. coli*. Furthermore, 9.4 % of these flocks were infected with IBV, NDV, and MG at the same time. Our data showed that the above mentioned respiratory pathogens were the most important cause of respiratory disease in broiler chickens in Haryana. Further studies are necessary to assess circulating strains. Farmers need to be educated about the significance and importance of these pathogens and their prevention through vaccination and other biosecurity measures.

Keywords: broiler, Haryana, respiratory pathogen

S3- 0197 First identification of the bacteria that causes spotty liver disease in layer birds in Australia

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The aim of this study was to identifying the aetiology of Spotty Liver Disease (SLD) in Australia. Spotty Liver Disease (SLD) is characterised by multiple, grey/white spots in the liver and causes significant egg production losses and mortality in free range and barn layer flocks, and broiler breeders housed in deep litter barns. Uncommonly it has also been seen in caged layers. The cause of the disease has long been a mystery but recently a putative SLD agent was identified in flocks in England and we now report that we have extended that finding to Australian chickens. A new *Campylobacter* spp. was isolated from the livers of 27 SLD affected birds from five disease outbreaks in Australia. The putative SLD agent was isolated from all outbreaks. Failure to isolate the bacterium from individual birds within an outbreak was mainly due to contaminants overgrowing the cultures rather than absence of the organism. A wide range of biochemical tests, electron microscopy and DNA sequencing were used to characterise the isolates and confirm that they represent a new species. The new *Campylobacter* spp. was characterised by colonies that were wet, creamy coloured, and could vary in size and morphology after long incubation times. They are Gram negative, motile, curved rods. The strains are catalase, oxidase positive, urease negative, and variable in hippurate hydrolysis and nitrate reduction ability. They were all resistant to nalidixic acid and metronidazole. The cells are curved rods with bipolar flagella. They have a low GC content and share identical 16S rRNA sequences with *Campylobacter* sp. FARM4 2011/1 isolate from England. The biochemical, structural and molecular characteristics of this bacterium confirm that this bacterium is a new *Campylobacter* species. Identification of the causative agent now opens the path to develop treatment options to protect chickens from SLD.

Keywords: spotty liver disease, *Campylobacter*, bird, chicken

S3-0198 Preparation and characterization of specific egg yolk immunoglobulin against *Edwardsiella tarda* in turbot (*Scophthalmus maximus*)

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Oral delivery of chicken egg yolk immunoglobulins (IgY) serve an interesting opportunity for controlling bacterial diseases in aquaculture and providing passive immunization of aquatic animals. This study evaluated the protective effect of IgY against *Edwardsiella tarda* infection in turbot. IgY was isolated from egg yolks laid by hens immunized with inactivated *Edwardsiella tarda*. The 2216E liquid medium containing different dosages of specific IgY (10 mg/mL, 5 mg/mL, 1 mg/mL) and non-specific IgY (10 mg/mL) were prepared, and medium with tetracycline (100 µg/mL) or no addition were used as positive and blank control respectively. 0.22 µm-filter sterilized solutions were added with *E. tarda* at a final concentration of 10⁶ CFU/mL. The mixtures were incubated at 28°C, and determined its absorbance at 600 nm by spectrophotometer at 4 h intervals. Purified IgY was shown to inhibit the growth of *Edwardsiella tarda* in vitro in a dose-dependent manner at concentrations from 1 to 10 mg/mL. The 96 healthy turbot were divided into 6 groups. Three groups of turbot were orally incubated with 50 mg/kg, 100 mg/kg and 200 mg/kg of sIgY respectively. All treatments were carried out 6 hours after intraperitoneal injection to turbot of all groups simultaneously with 100 µL of *E. tarda* at a concentration of 10⁸ CFU/mL. During the 10 days, mortality was recorded daily. And the bacterial load in liver, spleen, head kidney and intestine was investigated. The result shown that the bacterial load in turbot treated with the sIgY was significantly ($P < 0.05$) lower than those treated with non-sIgY. Moreover, preventive protection was shown in groups diet with 2% IgY powder and 10% egg yolk powder, which provided 75% and 62.5% survival rate, respectively and had dramatically difference compared to the negative control group ($P < 0.05$). These results suggested that passive immunization with specific IgY by oral intubation may provide a potential treatment for *Edwardsiella tarda* infection in turbot.

Keywords: *Edwardsiella tarda*, egg yolk antibody (IgY), turbot, bacterial load

S3-0199 Effect of melatonin on development of chicken small intestinal mucosa

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The concentration of melatonin (MT) in the intestine is much higher than the plasma and the total amount of MT in the intestine is around 400x higher than that in the pineal gland. Gastrointestinal MT is a multifunctional hormone, but little evidence has been demonstrated that MT can regulate the physiological functions of the intestinal mucosa. Here we treated the fertilized chicken eggs with MT (0.1-10 g) from E12 to post-hatching D6. The control group received PBS only. At D6, the small intestine was collected for morphological examination and gene expression determination. The morphologic features were identified by HE staining and AB-PAS staining, and the results showed that melatonin treatment significantly increased the number of goblet cells, but the enterocyte number was not changed. The cell proliferation was confirmed by Edu staining. We found that the number of proliferating cells and the distance of cell migration increased remarkably. In addition, we determined the activities of digestive enzymes and mRNA expression of the transporter proteins and ligand and receptor of notch signal by qPCR. The results showed that the activities of digestive enzymes and the gene expression of the transporter proteins were increased, while expression of the notch signals ligand and receptor mRNAs was depressed. The differential changes after MT treatment and the predicted targets suggest that MT may contribute to regulation of the intestine mucosa functions.

Keywords: melatonin, chicken, intestinal mucosa

S3-0200 Chicken's pericardial effusion—hepatitis syndrome outbreaks in parts of northern China and its quest for disease control and prevention

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In Shen county of Shangdong province, hybrid broilers's pericardial effusion-hepatitis syndrome outbreaks in July 2015. During the next four months, the disease epidemic to Henan, Jiangsu, Anhui, Hebei, Liaoning, Inner Mongolia, Shanxi, Shaanxi provinces. The disease infected with multiple varieties of chicken. Most mortality rates from 1% to 50%. Nine strains of the virus strains isolated from five provinces. Gene sequencing showed that these strains were adenovirus I group fourth serotype strains. Genetic evolutionary tree display besides further strain of Liaoningdalian (84.2%), others are highly approximation with strain of Shandongshencounty (> 98.8%). Relative to the Liaoningdalian strain, other strains's chicken embryos EID50 increased slightly, viral replication capacity 10 to 20 times. In animal regression test of ontology, the typical pathological symptoms appeared in 3 days after inoculation. Cohabitation chicken infected shows the spread of the disease also is likely to be limited infected with the air. Contain the virus in chicken embryo allantoic fluid can be inactivated vaccine. Immune two or three times, generally the serum antibody can reach 50ng / mL, it can protect the chicken from attack due to wild virus stains. If the antibody levels has reach 100 ng / mL, it's products can be used for the treatment of the disease. Advice that relevant departments support related basic research.

Keywords: chicken, adenovirus, disease

S3-0201 Identification of avian leukosis virus associated with high mortality in broiler flock in Malaysia

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Avian leukosis is an economically important retrovirus infection affecting meat and egg types poultry. The causative agent, avian leukosis virus is a single stranded positive sense RNA virus, which belongs to an Alpharetrovirus genus, family Retroviridae. Based on the envelope glycoprotein (gp85) it was possible to classify exogenous ALV into different subgroups namely A, B, C, D, E and J. An outbreak associated with high mortality suspected to be caused by viral infection was reported in a broiler farm of about 10,000-bird capacity in northern part of Malaysia. Samples were submitted to the virology laboratory for virus isolation and identification. Virus isolation in chicken's embryonated egg as well as in cell culture revealed evidence of virus growth, which was later identified by negative staining electron microscopy and confirmed to be an exogenous avian leukosis virus (ALV) using the next generation RNA sequencing approach. The identified ALV viral sequences were phylogenetically related to Taiwan and Japanese ALV strains and closer to subgroup J, subgroup E as well as recombinant A/E viruses. Based on these findings, ALV was concluded to be associated with the present outbreak. This study underscores the importance of exogenous avian leukosis virus in commercial broiler chickens in Malaysia and called for continued surveillance action for control and preventions

Keywords: avian leukosis virus, virus isolation, nextgeneration sequencing, broiler chicken, Malaysia

S3- 0202 Review of preventive and control strategies against histomonosis in turkey

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Histomonosis is a disease caused by a flagellate protozoan, *Histomonas meleagridis*. Complex life cycle of *H. meleagridis* can involve other parasites such as *Heterakis gallinarum* as an intermediate host, and many paratenic hosts like the earthworm. In turkey, the infection is manifested by typhlo-hepatic symptoms, in severe cases with high mortality and economic losses. The ban of Dimetridazole and Nifursol in 2003 was followed by an upsurge in reported cases and the disease re-emerged. In France, the disease increased from 1% to 6% of all parasitic diseases of turkey after 2003. Mortality varies from 10%, and can achieve 100% in some turkey holdings. The aim of this review is to focus on different preventive and control strategies against histomonosis in turkey. A positive effect of the paromomycin was reported for the treatment of this disease, but is not authorized in France, because its implication in the selection of resistant intestinal bacteria. With the ban of chemical molecules, the use of herbal medicine (Protophyt®, Natustat®, PrismaFlag®) and aromatherapy can be a prospect as alternative to treat histomonosis. The first results of herbal drugs are very encouraging, and should be supplemented by testing other "plant extracts" in experimental and clinical conditions. In relation to better treatment, it is important to improve the sanitary prophylaxis as well. It is currently the only available mean to limit the histomonosis in poultry. Several experimental immunization tests were conducted without success. Indeed, the attenuation of the pathogenicity of the parasite was not always accompanied by mitigating its immunogenicity; hence it failed to protect non-infested animals. With a lack of effective vaccine against histomonosis, vaccination remains a goal of future research.

Keywords: blackhead, *histomonas meleagridis*, treatment and control

S3- 0203 Introduction risk of a low pathogenic avian influenza virus in different Dutch poultry sectors

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Low Pathogenic Avian Influenza (LPAI) viruses of the H5 and H7 subtype have the potential to evolve to Highly Pathogenic Avian Influenza (HPAI) viruses in poultry and therefore infections with these subtypes are notifiable. Consequently, member states of the European Union have implemented surveillance programmes. In the Netherlands a syndromic surveillance and serological monitoring programme is in place. In the monitoring programme, all poultry farms are tested 1-4 times a year. Frequency differs between the different poultry types and housing systems (indoor and outdoor layer chickens, broilers, ducks, turkeys, etc) based on the supposed differences in the risk of introduction of LPAI infections. However, quantitative information regarding the possible differences in risk between these poultry types is sparse. In this study the rate of introduction of LPAI in different poultry types was quantified. Data from the Dutch LPAI monitoring program (2007 - 2013) were analysed using both generalized linear models and generalized linear mixed models, modelling the rate of introduction per year. By using these models the relative risk (RR) of introduction of LPAI per type of poultry farm, during the study period (years 2007 - 2013) could be quantified. Results showed that outdoor-layer farms had a 6.3, meat turkey 12.0, turkey-breeder 11.3, duck-breeder 25.5 and meat-duck 39.5 times higher RR of introduction of LPAI than indoor-layer farms. Layer-breeders, broilers and broiler breeders had a significantly lower RR of introduction of respectively 0.5, 0.2 and 0.4 times. If the analysis is restricted to the notifiable subtypes H5 and H7 RR for layer-outdoor, meat-turkeys and duck-breeders is even higher compared to indoor-layer farms. Differences in the risk of introduction of LPAI could be used to (re)design a targeted risk-based surveillance program.

Keywords: avian influenza, introduction risk, monitoring, Dutch poultry sectors

S3- 0205 Socioeconomics and drugs use perspectives of smallholder poultry farmers and animal health practitioners in Akwa Ibom state, south-south, Nigeria

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There is the need to understand the drug use habits of major stakeholders that drive emergence and sustenance of antimicrobial resistance (AMR) and production losses in the Nigerian poultry industry. Primary data generated from surveys of 59 poultry farmers (PFs) and 27 animal health practitioners (AHPs) in Akwa Ibom State, Nigeria were used to determine the variations in socio-economic characteristics, drug use/prescription habits and knowledge about AMR among these key stakeholders in the poultry industry. The study revealed high presence of female PFs (50.80%) in the area, while AHPs were mostly males (70.37%). Among these stakeholders, the 20 - 40 years age groups were predominant (74.02 and 83.04% respectively). About 45.00% of PFs had secondary education, while 40.67% had tertiary education (Animal science 27.27%, Animal health 22.72% and Agriculture 18.18%). Among the AHPs, 70.37% had tertiary education (veterinary 33.33%, animal health 29.62% and animal science 27.77%), while 29.62% had secondary education. About 72% of PFs retained the services of AHPs, and used mostly antibiotics (17.15%), among other drugs to medicate their birds. Antibiotics (14.28%) were also the most frequently prescribed drugs by the AHPs. There was high knowledge of AMR among the respondents (76.27% of PFs and all AHPs). Among the 15 and 13 diseases listed by PFs and AHPs respectively, AMR was encountered highest in cases of chronic respiratory disease (CRD) (23.40% and 19.44% respectively). The study also showed that abuse of antibiotic administration (14.01 and 12.10% respectively), poor dosage administration (4.01 and 12.10% respectively) and ignorance on the part of PFs (12.46 and 12.10% respectively) were viewed as the major production practice drivers of AMR in poultry pathogens. There is therefore the need to establish functional regulatory frameworks to help eliminate abuse of antibiotics by poultry farmers and animal health practitioners in Nigeria.

Keywords: poultry, antimicrobial resistance, animal health

S3-0207 PCR detection and characterization of *Enterococcus* sp. from commercial broiler chicken flock

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This study was carried out for the detection of *Enterococcus cecorum* from the first outbreak of Enterococcal- Associated Vertebral Osteoarthritis (EVOA) which was diagnosed by clinical signs, Gross and histopathological examination followed by isolation and identification of *enterococcus* sp. in pure culture from the femur and vertebral. The published primers of were used to carry out PCR followed by sequencing. Three isolated were detected positive by PCR. The 16S rDNA gene sequence was used to carry out BLAST and based on maximum identity score, most similar sequences were selected and aligned using multiple alignment software program, Clustal W. The nucleotide homology and blast result of the sample showed be to *Enterococcus* sp. The similar homologous species were found to be *E. gallinarum* strain FDAARGOS_163 (Accession no:CP014067.1) and *E. gallinarum* strain FMAC104 (accession no KF060264.1). *Enterococcus* species has been reported to be responsible for a number of debilitating conditions in humans such as infections of the urinary tract and also nosocomial infections, which could be life threatening in nature. This is the first study showing the presence of *E. cecorum* in Malaysia and its similarity with the other species in group which could provide the foundation for the future studies on antimicrobial resistance and molecular epidemiology of this particular isolate.

Keywords: *Enterococcus cecorum*, osteoarthritis, broiler chicken, PCR detection

S3-0208 Isolation, identification and characterization of one Nephropathogenic strain of infectious bronchitis virus in Malaysia

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Infectious bronchitis (IB) leading to ineffective production and mortality of chickens worldwide. This disease is caused by Infectious bronchitis virus (IBV), a member of family Coronaviridae. Frequent outbreaks of nephrotropic IBV has been observed in Malaysia and the renal damages has been observed in IB- vaccinated flocks, which suggested that currently commercially available IBV vaccines might not providing adequate protection upon challenge with a range of IBV field isolates and that outbreaks of disease can still occur within vaccinated flocks. Previous studies had showed that, avian coronavirus has ability to mutate and many of IBV strain have been reported. Thus, the objective of current project was to isolate identify and characterize IBV in Malaysia. The project was started from January until June 2015. Isolation of virus was done by inoculating IBV into allantoic cavity of ten days old embryo SPF eggs. The inoculated eggs were candled every day to check the death of embryo after the infection of virus. The infected eggs were harvested and RT-PCR was carried out from infected allantoic fluid for identification and characterization of virus. Molecular analysis revealed that 759 base pair (bp) was targeted band which is representing for IBV. These findings may lead us to develop an effective subunit vaccine against nephropathic IBV.

Keywords: isolation, identification, characterization, infectious bronchitis virus, Malaysia

S3-0209 Mapping of segmented filamentous bacteria in the intestinal tract of broiler chickens

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Segmented filamentous bacteria (SFB) are an unique group of commensal bacteria within the family of Lachnospiraceae, belonging to the order of Clostridiales. These bacteria are characterized by their attachment to the intestinal epithelium and their important role in modulating the host immune system. They induce IgA secreting cells and influence the development of the T-cell repertoire. In mice, several factors including age, intestinal segment, diet composition, antimicrobial drugs, immune status, and environmental stress factors, have been reported to affect the intestinal colonization of SFB. Recently, it was demonstrated that well-known predisposing factors for Clostridium perfringens induced necrotic enteritis in broiler chickens, such as coccidiosis and mycotoxins, reduce the intestinal abundance of SFB. The aim of this study was to map the normal intestinal distribution pattern and age-dependent occurrence of SFB in broiler chickens. An animal trial was performed with 132 non-vaccinated Ross 308 broiler chickens of both sexes, which were obtained as one-day-old chicks from a commercial hatchery. Birds were reared under standard husbandry and nutritional conditions for 42 days. Every two days, 6 birds (3 males/3 females) were euthanized and intestinal digesta and mucus samples were taken at different intestinal segments (duodenum, jejunum, proximal part ileum, mid-ileum, distal part ileum, caecum and colon). After the digesta were removed by gentle squeezing, the intestinal segments were opened longitudinally and rinsed with PBS. The mucosa was scraped with a microscope slide to collect mucus, epithelial cells and mucosa-associated bacteria. Intestinal digesta and mucus samples were snap frozen in liquid nitrogen and stored at -70°C. Subsequently, the number of SFB was determined by qPCR and normalized to the total number of intestinal bacteria (16S). Results will be presented at the conference.

Keywords: broiler, microbiota, segmented filamentous bacteria

S3-0210 Isolation and characterization of a distinct duck-origin goose parvovirus causing ducklings' short beak and dwarfism syndrome

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Since 2015, many mule duck and cherry valley duck flocks are suffering a new disease known as short beak and dwarfism syndrome (SBDS) in different duck producing areas of China, causing much economic losses to Chinese duck industry. This disease showed the typical symptoms as dyspraxia, weight loss, protruding tongues and feather disorder, accompanying by a high morbidity and low mortality. Five mule duck flocks and two cherry valley duck flocks were monitored for the SBDS disease in different districts of Fujian province of China. Serum specimens were collected for detecting MDPV and GPV antibodies by Latex Agglutination Inhibition assay (LAI) basing on MDPV and GPV special monoclonal antibodies. All SBDS ducks flocks were positive for MDPV and GPV antibodies with the sero-prevalence at 92%-100% respectively. Strikingly, the GPV antibodies titer was much higher than MDPV antibodies. Samples of SBDS ducks were positive for GPV specific antigen but not MDPV examined by LA and IFA assay. Liver, spleen and intestine of sick birds were homogenized for isolation and characterization. Eventually, one isolate named SB-GPV M15 was fortunately obtained after several blind passages' inoculation in SPF cherry valley duck embryos. The similar disease was successfully reproduced by inoculated mule ducks and cherry valley ducks with SB-GPV M15. All SB-GPV M15 inoculation groups were positive to GPV antibodies at 14-42 dpi and showed obvious similar symptom as natural cases. The beaks lengths of challenged group were obviously shorter than that of mock group ($P \leq 0.05$). The body weight of infected groups was strikingly less than the control group after 28-42 dpi ($P \leq 0.05$). A 348 bp (2604-2951) VP1 fragment was amplified and most closely related to Hungarian GPV strain which was also isolated from mule ducks SBDS disease. Together, these data indicate that SB-GPV M15 is one GPV related parvovirus accounting for SBDS and divergent from classical GPV isolates.

Keywords: short beak and dwarfism syndrome, isolation and characterization, goose parvovirus

S3-0211 Evolution of Newcastle disease virus quasispecies diversity and enhanced virulence after passage through chicken air sacs

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Wild waterfowl are generally considered to be natural reservoir of NDV, and the majority of the isolates are lentogenic or only potentially pathogenic. NDVs can be transmitted from wild waterfowl, via domestic waterfowl, to terrestrial poultry. Many studies suggests that the NDVs which have phylogenetic close relationship were circulating in domestic poultry and wild birds. RNA viruses exist in the host as a group of variants, known as a "quasispecies," a concept considered to play a critical role in pathogenesis. But there is no detailed analysis of NDV quasispecies yet, and the relationship between emergent virulent strains and NDVs quasispecies has not been clarified. Ultra-deep-pyrosequencing (UDPS) has emerged as an important tool with which to investigate viral diversity and to detect mutants in a group of quasispecies. The aim of the present study was to elucidate the quasispecies status on virulence effects by consecutive passages a duck-origin lentogenic isolate JS10 in chicken air sac. The velogenic properties of this selected variant was determined using mean death time (MDT) assays, intracerebral pathogenicity indices (ICPI), the intravenous pathogenicity index (IVPI), histopathology, and the analysis of host tissue tropism. By contrast, JS10 remained lentogenic after 20 serial passages in chicken eggs (JS10-E20). The JS10, JS10-A10, and JS10-E20 genomes were sequenced and found to be nearly identical, suggesting that both JS10-A10 and JS10-E20 were directly generated from JS10. And the partial genome covering the F0 cleavage site of JS10 and its variants were analyzed using ultra-deep-pyrosequencing (UDPS) and the proportion of virulence-related genomes in the quasispecies were calculated. Velogenic NDV genomes accumulated as a function of JS10 passaging through chicken air sacs. Our data suggest that lentogenic NDV strains circulating among poultry might be a risk factor to future potential velogenic NDV outbreaks in chickens.

Keywords: Newcastle disease virus, quasispecies, virulence evolution, ultra-deep-pyrosequencing

S3-0212 Studies on avian pathogenic *Escherichia coli* in commercial broiler chickens in Southeast Queensland

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To define and differentiate between avian pathogenic *E. coli* and (APEC) and avian faecal *E. coli* (AFEC) based on the association of a specific set of VGs, phylogenetic groups, antimicrobial susceptibility and plasmid profile and to determine the risk factors associated with APEC on the Australian broiler chicken farms. Fifty APEC from chickens with colibacillosis and 187 Avian AFEC from healthy chickens were subjected to antimicrobial susceptibility testing, phylogenetic grouping, plasmid replicon (PR) typing, clonality and virulence gene (VG) profiling. Isolates resistant to extended spectrum cephalosporins (ESC) and/or fluoroquinolones (FQ) underwent further characterisation. Representative isolates underwent extended VG profiling. Twenty-six percent of the AFEC and the APEC were susceptible to all antimicrobials. Resistance was most commonly detected to sulfamethoxazole/trimethoprim (39% AFEC and 44% APEC), Seven AFEC and three APEC were resistant to FQs and/or ESC. ESC resistant isolates contained blaCMY-2 or blaDHA-1 genes. The majority of the FQ resistant isolates were ST354. APEC isolates were 65 times more likely (95% CI 18.2 - 326) to contain the five virulence genes than AFEC isolates. The majority of AFEC and APEC were phylogenetic group A (16% and 50%) and C (28% and 16). The most common plasmid replicon types in AFEC and APEC were FIBa and Frep. The most prevalent APEC VGs were FeoB, iutA, iss, ompT, hlyF, sitA (>90%), vat (89%) and fimC (86%). This study revealed a set of genes that could be used to define APEC. Resistance levels of APEC and AFEC were similar and the first time detection of resistance to ESC and FQ, despite no history of use of these antimicrobials in the Australian broiler chicken industry, could point to sources external to the shed, especially since ST354 has been identified in several other sources.

Keywords: *Escherichia coli*, extended spectrum cephalosporins, fluoroquinolones, multidrug resistance, plasmid replicon, phylogenetic group, broiler, virulence genes, risk factors.

S3-0213 A direct evaluation of impact of 2 anticoccidial programs on performance and intestinal health, in a UK commercial broiler farm

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Intestinal integrity (I 2) in broilers is defined as the optimal functioning of the gastrointestinal tract. Where intestinal integrity can be maximised broilers are better able to express their genetic potential for performance; thus enhancing economic productivity. Coccidiosis remained one of the most costly threats to I 2 globally. Scientific management of coccidiosis by using ionophores or chemicals or vaccines is essential to maintain I 2 and profitability in poultry production. Mainstay of coccidiosis control in UK is ionophores like narasin and monensin. Although the mode of action of all ionophores is similar, several studies have shown that there are some significant differences like withdrawal period among ionophores that influence broiler performance. The study will be conducted to evaluate the influence of ionophores withdrawal period on performance and I 2 of broilers fed on either narasin (0 day withdrawal; evaluation group) or monensin (mandatory withdrawal; control group) in finisher rations. These experimental broilers were fed with potentiated ionophores (narasin + nicarbazin) in starter rations from 0 - 25 days. The study will span over 3 commercial crops in the defined facility covering nearly 2.5 million birds. Data will be available to summarise the findings of this study by 01/08/2016. The evaluation was carried out in a commercial farm of 16 sheds. 8 sheds each were allotted to control and evaluation groups. Average shed capacity is ~50,000 birds. We will disprove the null hypothesis, which will validate the statements below: The expected FCR improvement from a Maxiban-Elancoban program to a Maxiban-Monteban program is 3-5 points.

Keywords: anticoccidial, coccidiosis, intestinal integrity, coccidia, *Eimeria*, monensin, narasin

S3-0214 Kyt[®] SE DIVA 1 real-time PCR – an alternative and fast method for differentiation of Salmonella enteritidis live vaccine strain from field strains

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Live vaccination of chickens to protect against Salmonella Enteritidis (SE) field infection is of utmost importance in Salmonella control strategies. The detection of Salmonellae mostly is based on standardized cultural methods but may also be conducted with validated molecular methods that are used increasingly. The potential re-isolation and detection of SE live vaccine strains rather than field strains is a challenge to the diagnostician. So there is a need for methods capable of distinguishing between vaccine and field strains. There are cultural DIVA – Differentiating Infected from Vaccinated Animals – methods in place, but they are time consuming and need viable isolates. A DIVA real-time PCR detection method is presented which is capable to separately detect SE field and SE live vaccine strains in one reaction within 2 -3 hours after pre-enrichment. The method detects the live vaccine strain SE 441/014 (ade-/his-), which is present in commercial vaccines such as Salmovac SE, Salmovac 440 und Gallivac SE. Data and hands-on experiences derived from extensive validation studies are presented. In comparison to the cultural methods, the time for detection and differentiation of SE strains is reduced from 6 days to 30 hours. The system was found to be a fast and reliable alternative to the existing cultural DIVA method comprising even more informational value.

Keywords: real-time PCR, Salmonella Enteritidis, DIVA-PCR, Salmonella control strategy, Salmonella detection

S3- 0215 Comparative molecular, pathological and immunological studies between different circulating H9N2 AIV in Egypt

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H9N2 avian influenza virus considered major viral problem affecting poultry industry in Egypt since 2010 till now. The virus infection cause high economic losses in both layers and breeder due to drop in egg production also, sever losses in broilers was reported specially in case of co-infection of other viral or bacterial diseases. During late 2013 and early 2014 sever change in virus behavior as well as disease course have been reported in both broiler and egg producing chicken farms suggesting a change in the virus structure. Ten virus isolates were isolated from broiler (5 isolates), layer (3isolates) and broiler breeder (2 isolates) and partial hemagglutinin gene sequencing done indicating some nucleotide changes were reported in the isolated viruses. In comparison with old isolates of H9N2, the newly isolated viruses gave more sever tissue damage by histo-pathological examination and Immunohistochemistry. The results of cross protection study revealed that the locally produced vaccine from homologous strain of H9N2 give a better immune response against the new isolates of H9N2 viruses.

Keywords: H9N2 AIV, avian influenza, hemagglutinin

S3- 0216 Molecular detection and phylogenetic analysis of IBV in Egypt and the impact of locally produced inactivated vaccine

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In the present study 120 IBV suspected samples were collected from different governorates in Egypt during the year 2013, 2014 and 2015 (45, 40 and 35, respectively). The collected samples resembled different types of production (80 samples from broiler chickens, 25 samples from Layers and 15 samples from breeders). RNA extractions were done for the collected samples and undergo rRT-PCR where 87% (39 out of 45), 72.5% (29 out of 40) and 43% (14 out of 35) of the examined samples were positive during the years 2013, 2014 and 2015, respectively. Full length S1 gene for 12 selected (5 broiler, 3 layer and one 4 breeder) samples were amplified by end point RT-PCR and the products were purified using Montage DNA purification kit® and sent for DNA sequencing. The phylogenetic analysis of the selected samples revealed that 41.7% of the examined samples were genetically related to IS/885 strain while the remaining 58.3% of the examined samples were genetically related to IS/720-99. Field trials for evaluation of locally produced inactivated vaccine "ME VAC IB+ND" from homologous strains revealed 73% kidney protection in broiler chicks and 100% protection using the regular vaccination program in laying chickens.

Keywords: IBV, infectious bronchitis, vaccine, molecular detection

S3-0217 Efficacy of a bivalent inactivated oil-emulsion vaccine for avian influenza H9N2 and Newcastle disease viruses in chickens

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Significant role of avian influenza (AI) H9N2 and Newcastle disease virus (NDV) infections (single or mixed) was reported in terms of altering the severity of clinical signs and lesions in Egyptian poultry and further complicate vaccination programs. Therefore multivalent vaccines were suggested as a solution. In this study, a bivalent AI- H9N2 (Ck/EG/114940v/NLQP/11) and a virulent LaSota-like NDV (Ck/Eg/11478AF/11) inactivated oil-emulsion vaccine was developed. Doses of 350 and 400 hemagglutination units (HAU) for AI-H9N2 and NDV antigens, respectively, were mixed with Montanide-ISA70TM adjuvant. Vaccine sterility and safety was assured according to the OIE standards. The protective efficacy of developed vaccine was evaluated in 2 weeks-old SPF chickens received 0.5ml vaccine/bird subcutaneously. Hemagglutination inhibition (HI) antibody titers were monitored weekly. At 4 weeks post-vaccination (PV) birds were challenged with 10⁶ EID₅₀/0.1 ml of homologous AI/H9N2 and heterologous genotype VII virulent NDV (Ck/Egypt/567F/12) viruses separately via nasal route. The developed vaccine was well tolerated and induced protective HI antibodies by 4-weeks PV (8.5 log₂ and 8.2 log₂ for AI-H9N2 and NDV, respectively). A 100%, and 93.3% protection was observed in AI- H9N2 and virulent NDV challenge groups, respectively. Generally, virus shedding of both viruses was significantly reduced in vaccinated groups. The AI/H9N2 challenge group virus shedding was diminished by 3 days post infection (DPI). However, NDV virus shedding was significantly reduced by 6 DPI in terms of titer and number of shedders (2.6±0.7 in 4/14 birds). In conclusion, bivalent AI/H9N2 and NDV oil-emulsion vaccines could be used efficiently to protect commercial chickens from morbidity and mortality.

Keywords: avian influenza H9N2, AI H9N2, Newcastle disease, vaccine, avian influenza virus, efficacy, ai vaccine

S3- 0218 Development and evaluation of trivalent inactivated oil-emulsion vaccine for H5N1 and H9N2 avian influenza viruses

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Endemic highly pathogenic H5N1 (HPAI-H5N1) and avian influenza H9N2 (AI-H9N2) in Egyptian poultry has complicated the control efforts and vaccination programs. Multivalent vaccines were suggested as supportive tool for AI control. In this study, a trivalent reassortant HPAI- H5N1 (Ck/Eg/Q1995D/10-clade2.2.1.1 and Ck/Eg/M7217B/13-clade2.2.1.2) and AI- H9N2 (Ck/Eg/114940v/NLQP/11) inactivated oil-emulsion vaccine was developed. Doses of 450 and 350 hemagglutination units (HAU) for each H5 and H9 strains respectively were mixed with Montanide-ISA70TM adjuvant. Vaccine sterility and safety was assured according to the OIE standards. The protective efficacy of formulated vaccine was evaluated in 2 weeks-old SPF chickens received 0.5ml vaccine/bird subcutaneously. Hemagglutination inhibition (HI) antibody titers were monitored weekly. Challenge was conducted at 4 weeks post-vaccination (PV) using 106EID50/0.1ml of different HPAI-H5N1 (A/duck/Eg/M2583D/10-clade2.2.1, Ck/Egypt/1063/10-clade2.2.1.1, Ck/Eg/M7217B/13-clade2.2.1.2, and Ck/Eg/1575S/15-clade-2.2.1.2), and homologous AI-H9N2 viruses, separately via nasal route. The developed vaccine was efficient and induced protective HI antibody titers by 3-weeks PV (8-9 log₂ and 9 log₂ for HPAI- H5N1 and AI- H9N2, respectively). An 80%, 90%, and 100% protection were observed in clades 2.2.1, 2.2.1.1, and 2.2.1.2 viruses challenge groups, respectively. Virus shedding rate was reduced where only 20-30% birds remain virus shedders with significantly lower titers as compared to unvaccinated challenge group at 3 days post-infection (DPI), while only the clade2.2.1 challenged birds continued till 6 DPI. AI-H9N2 challenge group showed 100% protection and virus shedding was also diminished by 3DPI. In conclusion, the availability of new developed potent multivalent oil-emulsion vaccines could simplify the vaccination programs; and can be used as effective tool against AI control in endemic countries.

Keywords: HPAI- H5N1, highly pathogenic H5N1, AI-H9N2, vaccine, avian influenza virus

S3-0219 Protective efficacy of combined vaccines against Newcastle disease and infectious bronchitis viruses in layers chickens in Egypt

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A study has been carried out to evaluate different local and imported combined inactivated NDV+IBV vaccines and investigate its ability to protect experimental broiler chickens against challenge with wild ND and IB viruses. Two hundred and eighty commercial 14 weeks-old layer Saso chickens were divided into 8 groups (35 birds each). Groups 2, 3, 4 & 5 were vaccinated at 14 weeks old with live hitchner + IB Primer. Then all groups at 18 weeks old were boosted with inactivated combined NDV + IBV vaccines with different adjuvants except for group 1 & 2. Group 1 was kept as unvaccinated control. At 20 weeks old all groups was challenged with a dose of 105.5 EID50/bird of Variant 2 genotype (1494) IBV through oculo-nasal rout. Blood samples were collected from 10 birds selected randomly from each group at weeks 14, 15, 16 till 22 for sera separation. Organs of 3 birds/each group were collected (trachea, bronchi, kidney and oviducts) for PCR at 3,7,10 days post challenge for antibody titer monitoring by HI and ELISA. Groups 3 & 4 showed high protection with all serological tests. Based on our findings ISA 71 proved to be a good adjuvant as the groups received the inactivated vaccine which contain it as booster after priming with live vaccination, showed high and good protection against IBV especially after challenge with wild live IBV virus where the virus couldn't be detected in trachea & kidney even by using the sensitive rRT-PCR.

Keywords: infectious bronchitis disease, Newcastle disease, layer chickens, inactivated combined NDV+IBV vaccines, ISA70, ISA 71, protection

S3- 0220 Avian influenza virus vaccine match against circulating H5N1 avian influenza viruses in Suez Canal region, Egypt

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Different types of inactivated imported and locally produced AI vaccines have been used in Egypt in order to control the AIV infection in poultry and regardless the countrywide control plan and vaccination policy of poultry to stop the widespread of HP H5N1 AIVs, continuous circulation and evolution of the viruses in all poultry sectors is still recorded every season. This reflects the need to review efficacy of vaccination strategy and commercially available vaccines used in the field. Therefore, in this study, samples were collected from different poultry production sectors in Suez Canal region during 2014-2015 then viral RNA was extracted and subjected to real time reverse transcription polymerase chain reaction (RT- qPCR) then positive samples were further subtyped for detection of the H5 gene then sequence analysis for HA gene and sequence similarity to match the recently circulating Egyptian viruses against the currently used imported and local vaccines seed strains. Our results revealed that the amino acid identity percent of vaccine seed strains of American and European origin showed the lowest similarity (76%- 77%) while the Chinese strains showed higher similarity (91%- 92%). However, the highest amino acid identity percent was observed with Egyptian seed strains (93%- 99%). Consequently, our study verified that there are wide range of genetic similarity of the Egyptian vaccine seed strains against the currently circulating field viruses in Egypt, this reflect the importance of continuous evaluation and updating of the vaccine seed strains to determine its efficacy against the currently circulating field virus.

Keywords: Avian influenza virus, vaccine match, H5N1, Suez Canal region, Egypt

S3- 0221 Salmonella Heidelberg resistance profile isolated from poultry houses in Brazil

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Salmonella Heidelberg resistance profile isolated from poultry houses in Brazil. Universidade Estadual Paulista “Júlio de Mesquita Filho”, São Paulo, Brazil. Corresponding author: andreatti@fmvz.unesp.br In recent decades, there have been changes in the prevalence of Salmonella serotypes associated with poultry and humans. Currently, the serovar Heidelberg has caused deaths in the US and other countries. This study aimed to evaluate the resistance profile of strains of Salmonella Heidelberg (SH) isolated from poultry houses. We evaluated SH 37 samples isolated from slaughter houses and poultry farms of Brazil. To evaluate the acid resistance, samples were subjected to pH 2.5, sampling was performed prior to acidification (t=0) and after 2 hours at pH 2.5 (t=1), further cultivation in Brilliant Green Agar. The survival rate (SR) was calculated as follows: > 10%, high acid resistance, < 0.01% low acid resistance, the others were considered intermediate. The sensitivity test to antimicrobials was performed by disk diffusion method in Müller-Hinton agar. The antimicrobials used were amoxicillin + clavulanic (30µg), ampicillin (10µg), ciprofloxacin (5µg), sulfamethoxazole trimethoprim (25µg), doxycycline (30µg), streptomycin (10µg), gentamicin (10µg), norfloxacin (10µg) and tetracycline (30µg). Only one isolated SH showed high acid resistance (15.7%), 26 showed intermediate resistance and 10 had low acid resistance. All isolates were resistant to one or more antimicrobials, and three (8.1%) of them were resistant to seven of the eight antibiotics tested. Twenty isolates (54%) were resistant to four or more antimicrobial classes. The majority of isolates (92%) were resistant to tetracycline, ampicillin and amoxicillin+clavulanic acid. The resistance profile of the samples, coupled with the propensity of serovar in causing invasive infections, make SH a major concern in the Brazilian public health. Keywords: antimicrobials, resistance, Salmonella Heidelberg, poultry

Keywords: antimicrobials, resistance, Salmonella Heidelberg, poultry

S3-0222 QXL87, the first attenuated live infectious bronchitis virus vaccine licensed to clinical trials in China

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Infectious bronchitis (IB) is an acute, highly contagious disease of chickens caused by infectious bronchitis virus (IBV). QX-like IBV is a new variant serotype firstly found in a flock of chickens suffering with proventriculitis in China. Currently, QX-like IBVs have been reported in most Asian and European countries. However, no pe-cific vaccines are yet available in China. Here, QXL87, an attenuated QX-like IBV strain is described. The field isolate, QXL, was passaged 120 times in embryonated SPF chicken eggs. The complete genome of different passages were sequenced, and 154 nucleotide substitutions were identified in the whole genome from 5th to 87th passage, which resulted in 56 amino acid substitutions and one nucleotide insertion in the putative ORFX. Most variation occurred before 60th passage (54/56), and main substitutions were located in nsp2 and nsp3 coding regions. In order to evaluate the pathogenicity of passaged virus, different passages were selected to challenge 1-day-old SPF chickens. The 10th-30th passages showed 80% morbidity and 20% mortality, respectively. The pathogenicity of the 50th passage was significantly decreased and morbidity was only 20%. No clinical signs could be observed in chickens inoculated virus after 70th passage, and no lesions were found in respiratory tract and kidney. Passage level 87 was selected to test the efficacy in SPF chickens. The vaccination-challenge test showed that the QXL87 passage could afford more than 90% protection efficacy against virulent homologous virus. The QXL87 was further tested for efficacy in commercial chickens with maternally derived antibodies, and it was also efficacious against challenge. In conclusion, the attenuated QXL87 strain might be a promise vaccine candidate for the prevention of QX-like IBV infection. Based on the QXL87 strain, a bivalent vaccine (NDV La Sota strain + IBV QXL87 strain) has been developed and licensed to clinical trials by Ministry of Agriculture of China.

Keywords: infectious bronchitis virus, QX-like, attenuated vaccine

S3- 0223 Toxicopathological effects of moldy feed in white leghorn hens and their amelioration with bentonite clay

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Mycotoxins are secondary metabolites of toxicogenic fungi. There are different mycotoxins which, gives different detrimental effects. The present study is designed to examine the adverse effects of feeding of moldy feed on White Leghorn(WL) layer hens and their amelioration by concurrent feeding of bentonite clay. Bentonite clay in different concentrations (0%, 0.5%, 1%, 2%) was administered alone and concurrently with moldy feed for a period of 16 weeks. The feed was analyzed for Aflatoxin B1 and Ochratoxin A level. Their blood samples were collected for different parameters, including serum biochemistry and immunological parameters including PHAp, CCA etc. Tissue samples were collected for histopathology. The data thus obtained was statistically analyzed by Analysis of variance test. The results showed that there was a significant decrease in the body weights of the hens in the group fed moldy feed alone, while their weight remain stable in the groups fed bentonite. Serum biochemical parameters were significantly high in group fed moldy feed alone and significantly less in combination groups. Egg weight was significantly high in groups given bentonite. Egg production was significantly high in the groups given bentonite at different levels. The current study showed that moldy feed when given to birds resulted in decreased feed intake, egg weight, egg production and had a negative effect on immunological parameters while these changes were not seen in groups given bentonite clay which indicated ameliorative effects of bentonite clay against moldy feed in a dose related manner.

Keywords: Aflatoxins, Pathology, Liver, Kidney, Mycotoxins

S3- 0224 Selection of oil adjuvants for production of vaccines against poultry pasteurellosis

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Scientific-production enterprise AVIVAC produces a wide range anti-viral and anti-bacterial vaccines, including vaccine against pasteurellosis, which proved its efficiency against all serotypes of *P. multocida*. In this work we aimed to select an adjuvant, which could enable an optimal antigenic activity with lowest reactogenicity of the anti-pasteurellosis vaccine and develop recommendations for efficient immunization. For production of a vaccine' samples there was used inactivated soluble antigenic complex *P. multocida* strain «115» and adjuvants, produced by the company SEPPIC: Montanid ISA 70 VG; Montanid ISA 71 R VG; Montanid ISA 775 VG; Montanid ISA 201 R VG and ESSAI 1113106. There were tested 5 vaccine samples, containing equal antigenic quantity in one immunizing dose. The vaccine samples were sterile, stable, with viscosity within 27 – 74 mm²/s². Antigenic activity and degree of reactogenicity were tested on Hysex White chicks in age 30 days. Each group was immunized by experimental samples of vaccine in dose 0,5 sm³. Additionally, each group was divided into 3 subgroups. In subgroup "a" vaccine was injected subcutaneously into lower third part of the neck; "b" – subcutaneously into breast area, "c" – intramuscularly into breast. To define antibody titer to *P. multocida* there were taken serum samples one day before and 30 days after immunization and tested by IFA method on IDEXX test-systems. There were found, that the most efficient and harmless was the vaccine against pasteurellosis, produced on the base of adjuvant Montanid ISA 70 VG. Intramuscular injection displayed highest antigenic activity and lowest reactogenicity.

Keywords: chicken, vaccines, pasteurellosis, adjuvants, injection

S3- 0225 Preliminary evaluation of the combined effect of protease and protein content in the diet on broiler caecum microbiota

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This study examined the effects of the dietary supplementation of proteases on the caecum microbiota of broilers fed with diets characterized by different protein levels at 14 and 42 days of age. A total of five caecal contents were collected at each sampling time from the following groups: (1) control group (i.e., basal diet); (2) group fed with the basal diet - 7% protein; (3) group fed with the basal diet - 7% protein and supplemented with protease; (4) group fed with the basal diet - 4% protein and supplemented with protease. Total DNA was extracted from each sample and submitted to shotgun metagenomic sequencing. The results of caecum contents of the bird belonging to the group 4 were significantly different in comparison to the other groups showing the possibility to modulate the gut microbiota modifying the diet. Therefore, the impact of the combination between decrease of protein content and the supplementation of protease should be further investigated.

Keywords: metagenomic analysis, caecum microbiota, protease, protein, broiler

S3-0226 Impact of *Lactobacillus acidophilus* d2/csl (CECT 4529) supplementation on broiler caecum microbioma and metabolic functions

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This study examined the effects of the dietary supplementation of *Lactobacillus acidophilus* D2/CSL (CECT 4529) on broiler caecal microbioma and metabolic functions. A total of 14 caecal contents were tested. Four samples were collected at day 0, before dietary treatment. Moreover, five samples were collected from both control and treated groups at 41 days. At the end of the rearing period the relative abundance (%) of Lachnospiraceae was significantly higher in treated birds in comparison to the control (17.07 vs 14.39; $P=0.036$). The bacterial species significantly higher in the chickens treated with 1×10^9 CFU/kg of *Lactobacillus acidophilus* were *Ruminococcus obeum*, *Clostridium clostridioforme*, *Roseburia intestinalis*, Lachnospiraceae bacterium 14-2T and *Coprococcus eutactus*. Part of these species belongs to *Clostridium* cluster IV, positively impacting gut health through the production of butyrate and short chain fatty acids. The metabolic functions significantly higher in the treated group at 41 days belong to the biosynthesis of other secondary metabolites, glycan biosynthesis and metabolism, folding, sorting and degradation, carbohydrate as well as amino acid metabolisms. Particularly interesting is the higher level of the β -glucosidase contributing to the hydrolysis of glucose monomers from non-starch polysaccharides playing an important role in the fermentation of undigested carbohydrates and ultimately in animal performance and health.

Keywords: *Lactobacillus acidophilus*, metagenomic analysis, microbioma, metabolic functions, gut health

S3- 0227 Epizootologicheskoy monitoring of flu of a domestic and wild bird in the territory of Sverdlovsk region

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Relevance and the practical importance of a problem of an avian flu dictate need of broad approach to epizootologicheskoy monitoring that will allow to expect tendencies and regularities of development of epizootic process, to develop scientifically based programs of elimination and not to allow distribution of this disease.

Keywords: avian flu, epizootologicheskoy monitoring, diagnostics, laboratory monitoring

S3- 0228 *Clostridium perfringens*, Shigatoxigenic and Enteropathogenic *Escherichia coli* from commercial poultry farms in Brazil

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Nowadays, the Brazilian poultry industry is the most important exporter in the world and the third one in production with 12.69 million tonnes, only behind USA and China. Among this, Brazilian production of chicken meat, 95% was destined to the production as raw meat, and only 5% was processed in poultry industries. Even with high technology in production, sanitary sector is still a challenge. *Clostridium perfringens* and *Escherichia coli* infections are considered a major problem in this industry, and to prevent these contaminations in the poultry chain, a bacteriological monitoring must be made with the elimination of infected birds in farms and industries. This study aimed to detect, by PCR multiplex, the presence of *C. perfringens*, shigatoxigenic *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC) in commercial poultry farms. For this research, 164 samples were collected from the chicken's cloaca using sterile swabs, which were subjected to bacterial culture in 5 mL of Brain Heart Infusion (BHI) broth, incubated at 37°C for 24 hours. The clostridia samples were incubated under anaerobe conditions. After growths, all samples were submitted to DNA extraction by boiling method and subsequently submitted to multiplex PCR with specific primers. *C. perfringens* was detected by the amplification for *cpa*, *cpb*, *etx*, *iap* and *cpe* genes and *E. coli* by the amplification of *eae*, *stx1* and *stx2* genes. The genes *stx1* and *stx2* were used to identify principally STEC, and the gene *eae* for EPEC. Bacterial identification by PCR resulted in 107 positive samples for EPEC, which amplify only the *eae* gene. The positive samples of EPEC were 65.24%. None sample was identified for *C. perfringens*. Thus, these results showed that is important to do a proper management in farms and industries to prevent the spread of pathogenic bacteria and the contamination of final products that will be consumed by humans, and may cause damage to public health.

Keywords: poultry, molecular biology, Pathogens

S3- 0229 Molecular characterization of nonstructural protein 15 of infectious bronchitis virus

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The genome of Infectious bronchitis virus (IBV) encodes 4 structural proteins and 15 nonstructural proteins (nsp2-16). Study on nonstructural proteins of other coronavirus such as SARS-CoV was carried out widely. However, few studies on IBV nsps are reported. The purpose of this study is to characterize IBV nsp15. IBV nsp15 gene was amplified from isolate SC021202 by RT-PCR and then expressed in *E. coli*. Purified recombinant nsp15 was used as antigen to prepare monoclonal antibody to nsp15, and subjected to analysis of its enzyme activity. Single amino acid site mutants were constructed and expressed in *E. coli* to determine the key activity sites of nsp15. Finally, to analysis the antigenic epitope of nsp15, truncated nsp15 fragments fused with EGFP were constructed and transfected DF-1 cells. The expression of truncated nsp15 in transfected cells was detected by IFA with the mAbs. Recombinant IBV nsp15 was expressed in *E. coli*, which was recognized by IBV-positive serum in WB analysis. Two mAbs to nsp15 were obtained which reacted with native nsp15 in both IBV infected and nsp15 transfected cells by IFA. H223, H238, K278 mutations enhanced the expression of nsp15 in *E. coli* greatly, but led to lose the enzyme activity of Endoribonuclease. The epitopes of nsp15 was found locating between 66-81aa and 214-223aa, respectively. The full length of nsp15 evenly distributed in the whole transfected cells, and the domain II (151-212aa) is responsible for this diffusion expression of nsp15. While the expression of domain I (1-150aa) and domain III (213-338aa) displayed as speckles in the cytoplasm. The expression pattern of IBV nsp15 is different from MHV. The domains of IBV nsp15 have distinctive expression pattern in the cells which is different from that of other coronavirus. The data present here might be useful for further understanding the pathogenicity difference among coronaviruses.

Keywords: IBV, nsp15, molecular characterization

S3- 0230 Efficacy of Flavomycin® and B-Act® administered via feed in controlling experimentally induced necrotic enteritis in broilers

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Necrotic enteritis, caused by enterotoxigenic *Clostridium perfringens*, affects the gut health of industrial poultry worldwide. Antimicrobials or probiotics can be vital to cure or prevent the disease. In this floor pen trial, broilers were supplemented with either Flavomycin® (32 ppm Flavophospholipol) or B-Act® (6.4 x 10⁶ colony forming units *Bacillus licheniformis*/g feed). Necrotic enteritis was experimentally induced by feeding a high protein diet, a coccidiosis challenge and *Clostridium perfringens* inoculation. Next, zootechnical performance and intestinal lesion scores of the supplemented birds were compared with an infected untreated control (IUC) group. The mean body weight was numerically higher but not significantly different in Flavomycin® and B-Act® treated birds compared to the IUC group. However, the proportion of birds with necrotic enteritis tended to be lower in the Flavomycin® group compared to the IUC group. Additionally, B-Act® supplemented birds were less likely to show higher necrotic enteritis lesion scores compared to the IUC group. In conclusion, under the present study conditions, Flavomycin® and B-Act® reduced the pathology caused by experimentally induced necrotic enteritis.

Keywords: broiler, probiotic, necrotic enteritis, B-Act, gut health

S3- 0231 Deglycosylation at site 158 in hemagglutinin affects the receptor binding property and transmissibility of clade 2.3.4 H5 subtype avian influenza virus

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Currently, novel subclade 2.3.4.4 within clade 2.3.4 H5 subtype avian influenza viruses of various NA subtypes pose a substantial threat to poultry industry, and most of which possess affinity to both α -2,3 (avian-type) and α -2,6 (human-type) receptors. To identify the key amino acids rendering the dual receptor binding property, site-specific mutants were generated on the genetic backbone of a clade 2.3.4 virus with only α -2,3 affinity via reverse genetics, based on the differentiated residues selected by hemagglutinin (HA) gene sequence alignment with the reported H5NX viruses owning dual receptor affinity. HA assay using goose red blood cells pretreated with α -2,3-specific sialidase and the solid-phase direct binding assay were applied to determine the receptor binding variation of those mutants. The results showed that T160A resulting in the lack of an oligosaccharide side chain at site 158 of HA, was responsible for the recognition of the α -2,6 receptor. In addition, the deglycosylation contributed to the transmissibility of the mutated clade 2.3.4 H5 virus among guinea pigs. Collectively, our results suggest that the elimination of glycosylation at site 158 may serve as an important molecular marker for assessing the pandemic potential of clade 2.3.4 H5 isolates.

Keywords: avian influenza, clade 2.3.4, glycosylation, receptor binding, transmissibility

S3- 0233 Effect of mixing Acidal® with drinking water for laying hens on Salmonella, total germs and production performance

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The prohibition of antibiotics used as growth factors since 2006 affects animal performance and economical viability of farms. Several alternatives including incorporation of organic acids, essential oils, probiotics and prebiotics in feed or drinking water in order to improve productivity were studied. The objective of this study is to mix "Acidal®" with drinking water of laying hens in order to control salmonella and improve productivity. The experiment was carried out on 360 ISA Brown hens (22 to 44 weeks of age), allocated to 3 groups of 120 pullets each. These groups were 1) control group (cont), 2) group that received 1mL of Acidal per liter of water (Aci1) and 3) group that received 2mL of Acidal per litre of water (Aci2). For each group, the chickens were divided into three replicates of 40 pullets each. Prior to start, every four weeks, samples of chicken droppings according to each group were collected and used to determine total Streptococcus and Escherichia coli and to check the presence of Salmonella. During treatments, quantity of water consumption, feed consumption, body weight, and egg component weights were recorded weekly. Number of eggs produced was recorded daily and every two weeks, the litter quality was assessed. Mixing of Acidal with drinking water of laying hens reduced significantly the number of total bacteria, eliminated completely Salmonella germs in the droppings and decreased feed intake but had no effect on water consumption, mortality rate, egg laying rate and the ratios of albumen, yolk or shell weights to egg weights. Acidal treatment improved egg weights and body weight compared to control group.

Keywords: antibiotics, growth promoters, alternatives, organic acids, Acidal® ML

S4- 0001 Effect of semen collection procedure on fertility rates of ostrich (Struthio camelus)

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Abstract This study was conducted to investigate the effect of semen collection method on fertility and hatchability rates and sperm penetration holes of ostrich. A total of 9 males and 27 females, 3 - 7 years old were randomly assigned to three treatment groups with 3 males and 9 females for each treatment group. Three semen-collecting methods were tested: (1) manual massage method; (2) dummy method; and (3) teaser female method. In all collecting method fertility and hatchability rates and sperm penetration holes were assayed for three consecutive months. Results revealed that the use of teaser female or dummy methods for collecting the semen from male ostrich resulted in significant increase ($p \leq 0.05$) with respect fertility (%), hatchability from fertilize eggs (%), hatchability from set eggs (%), and sperm penetration holes and significant decrease ($p \leq 0.05$) concerning embryonic mortality (%) in compare with manual massage method during all three months of experiment and as regards the total mean of these five traits. Development of the animal friendly methods (teaser female and dummy methods) for collecting semen from ostriches has advanced considerably in recent years. Normal ejaculates can be collected regularly but the males need to be trained and important human- bird interaction stimulating birds to perform sexual behavior need to recognized and taken advantage of in training. Semen collected by the teaser or dummy method is of good quality and quantity and is suitable for storage and gave very good results with relation to fertility and hatchability rates and sperm penetration holes. The ostrich industry appears to be in a good position for development of the artificial insemination technology although adoption of the artificial insemination technology in ostrich breeding will mean substantial changes to the current industry structure.

Keywords: Semen collection procedure, fertility and hatchability rates, ostrich.

S4-0002 Effects of gonadotropin-inhibitory hormone on steroidogenesis and apoptosis in goose ovarian granulosa cells

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Background: Gonadotropin-inhibitory hormone (GnIH) is a hypothalamic dodecapeptide hormone. It appears to act as a key neuropeptide involved in the control of vertebrate reproduction. Previous results indicated that GnIH and its receptor are expressed in the vertebrate ovary, and suggested a potential role for GnIH in controlling gonadal development and germ cell maturation. But it is not known about how GnIH worked directly on the tissue of ovary. This study was conducted to identify the physiological functions of GnIH in goose ovarian steroidogenesis and apoptosis. **Methods:** Goose (*Anser cygnoides domesticus*) ovarian granulosa cells were dissociated, cultured in vitro and treated with different doses of quail GnIH. We also assessed the concentrations of estradiol (E2) and progesterone (P4), cell numbers, and the expression levels of StAR, CYP19, 3beta-HSD, Bcl-2, CCND1, and CCND2. **Results:** Treatment with GnIH significantly decreased the E2 and P4 concentrations, and downregulated the relative gene expression levels for StAR, CYP19 and 3beta-HSD, all associated with the synthesis of steroid hormones. GnIH treatment significantly decreased cellular viability with decreases in the percentage of BrdU-positive cells and increases in TUNEL-positive apoptotic cells, and downregulated the levels of genes associated with cell proliferation: Bcl-2, CCND1 and CCND2. **Conclusions:** GnIH inhibits goose ovarian function by inhibiting steroidogenesis and promoting apoptosis in granulosa cells.

Keywords: gonadotropin-inhibitory hormone, goose ovary, granulosa cells, steroidogenesis, apoptosis

S4-0003 Egg production in the Progeny of transgenic quails

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Transgenesis allows obtaining of animals with modified traits which could be used in practical selection. Transgenic quails were obtained by microinjection of exogenous DNA into germinal discs of oviducts. Injected genetic construct contained gene of metallothionein-I promoter (mice) and structural gene of bovine growth hormone. Transgenic quails were detected by hybridization analysis of DNA blots and by PCR technique. Analysis of transgene expression was performed at the protein level (ELISA analysis of bovine somatotropine concentration in blood serum). To evaluate productivity of transgenic quails the control group (non-modified Estonian breed, 33 generations) and experimental group (progeny of transgenic Estonian quails, 33 generations) were compared. Generations consisted of 45-110 individuals each. In transgenic quails and their progeny egg weight was 10-20% higher compared to control. Egg production in several generations of experimental group was 200-235 eggs during 10 months of reproductive season while in control 193-227 eggs. Average individual yield of egg mass during reproductive season in transgenic group (2730.3 g) was 10% higher compared to control. This correlation was found in all generations studied. Concentration of lipids in egg yolk in experimental group was 26.1-29.3% vs. 27.8-29.2 in control. Protein content in egg yolk was 15.1%-17.0% regardless group, generation, and egg weight. No regular difference between groups was also found in water content in egg yolk. Egg white in both groups contained 85.0%-87.9% of water and 8.9%-10.9% of protein, with no difference between groups and generations.

Keywords: transgenic quails, eggs, bovine somatotropin

S4-0004 The tissue localization and inhibition of avian leukosis virus subgroup J by avian β - defensin 6 (AvBD6)

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Recently, With the reasons of the intensive and high-density rearing of poultry, a great numbers of global diseases have been broken out, such as ALV-J, MDV, REV and so on. ALV-J, characterized by medulloblastoma and other cell malignancy, is a sort of Infectious neoplastic disease. Since ALV- J was found in China, with the growth of infection host ranges and the advance of onset ages, the types of tumor increase sharply, that has brought serious economic losses in poultry. The main structure features of AvBD is six cysteine residues that form three pairs of disulfide bonds. AvBD is the first defense of microbial invasion in body. With broad-spectrum antibacterial, anti-viral and anti-fungal biological effects, AvBD plays an important role in the innate immune system in poultry. To further study the distribution in tissues and anti-virus effect of the chicken AvBD, we use PCR methods, amplified AvBD6 genes from chicken liver tissue. The sequencing result was to be amino acid sequence homology analysis with avian β - defensins and bird β -defensins, drawing molecular phylogenetic tree. The results showed that AvBD6 gene precursor peptide by is 67 amino acid residues, and the cDNA is 204bp, comprising six cysteine stable, located in 1-5,2-4,3-6 and linked to form 3 molecular disulfide bonds, inverted form a stable parallel 3 shares β - sheet structure C-X4-8-C-X3-5-C-X9-13-C- X4-7-CC, The highest amino acids homology is AvBD7 up to 62.7%.Chicken AvBD6 was subcloned into the expression vector pGEX-6p-1, and the recombinant plasmids were transformed into Rosseta (ER2566) competent cells, induced by IPTG. Expression products of chicken AvBD6 fusion protein is present as inclusion bodies. A preliminary study of inhibiting the ALV- J viral infection by AvBD6 recombinant protein in vitro can lay the foundation of the study of the biological function of AvBD and the innate immune system of avian.

Keywords: chicken avian β - defensin6, ALV- J; Real-time fluorescent quantitative PCR

S4-0005 Optimization of deep-freezing method of early embryonic cells of Guinea fowl (*Numida meleagris*) for gene preservation purpose

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Supporting the conservation of valuable indigenous domestic animal species is an important part of the new Hungarian agricultural strategy. In case of poultry species it would take six generations to return to the desired genotype after semen cryopreservation. The alternative approach may be the use of embryonic cell cryopreservation followed by re-implantation into host embryos. The blastodermal cells lead to the production of germline chimera which is able to recover the whole genome after one generation. The aim of the study was the development of an optimal deep freezing protocol for Guinea fowl blastodermal cells for gene conservation purposes. The effect of two different collection temperatures (+20°C; +4°C), two cryoprotectant combinations (10% DMSO; 5% DMSO + 5% EG) and two types of containers (straws; cryovials) were compared respectively on the survival of BCs. The survival rate of cells was tested at collection, after the addition of cryoprotectants and at after thawing. The tests were 8 times separately repeated. During the experiment non incubated fertile eggs in stage X-XII were used and embryonic cells were collected on two different collection temperatures from the blastoderm. The cell suspension was cleaned, halved and two different cryoprotectants were added. Straws and vials were filled with the differently treated cell suspensions and a slow freezing protocol was used. The straws and vials were stored in liquid nitrogen on -196°C. Thawing was carried out in 27°C water bath. The collected data were analysed with statistical methods. Results: The collection of embryonic cells on +4°C has caused significantly higher results compared to collection at room temperature. After thawing of the samples the best survival rate (71%) was found in case of cells collected on ice and stored in vials with a cryoprotectant containing 5% DMSO + 5% EG. The newly developed protocol is suitable for the long term preservation of Guinea fowl blastodermal cells.

Keywords: Guinea fowl, avian, blastodermal cells, slow-freezing

S4- 0006 Ontogenic gene expression of leptin receptor and ghrelin receptor in the pullets' ovary

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Abstract Leptin and ghrelin have been implicated in reproductive development especially the attainment of puberty. The association of the expression of the genes with reproductive development is lacking in poultry species especially with the controversy on the authentic leptin gene in birds. The ontogenic expression for leptin receptor (LEPR) and ghrelin receptor (GHSR) genes in the pullets' ovary were studied. A study of the receptors may provide some insight into the activities of the genes. Two birds were slaughtered and their ovaries aseptically removed at week 6, 8, 10, 12, 14, 16, 17, 18, 19, 20 and 21. Total RNA was extracted, followed by RT-PCR analysis and then gel electrophoresis. The bands were then semi-quantitatively analysed using band densitometry. The results showed that both LEPR and GHSR genes were expressed in the chicken ovary during early developmental stages until puberty. However, densitometric analysis of expression levels did not show significant changes in expression levels throughout the period of sampling. These findings affirm that there may be a role for both leptin and ghrelin in the ovary of chickens but the mechanisms of their action on the attainment of puberty may not involve the level of gene expression of the receptors.

Keywords: : leptin receptor (LEPR), ghrelin receptor (GHSR), pullets, ovary, Reverse transcription- Polymerase chain reaction (RT-PCR), Puberty

S4- 0007 Cytotoxicity of various chemicals and mycotoxins in fresh primary duck embryonic fibroblasts: a comparison to HepG2 cells

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To cost-effectively screen the overall toxicity of a sample, especially in the case of food and feed ingredient quality control, a sensitive bioassay is necessary. With the wide variety of cytotoxicity assays, performance comparison between assays using different cells has become of interest. Fresh primary duck embryonic fibroblasts (DEF) were hypothesized to be a sensitive tool for in vitro cytotoxicity screening; cell viability of DEF in response to various cytotoxins was determined and compared with response of HepG2 cells. The IC50 values obtained by alamar blue assay in DEF cells had high correlation ($r^2 = 0.96$) with those obtained in HepG2 cells. Within the same toxin, primary DEF yielded significantly lower IC50 values than that obtained from HepG2 cells using the MTT and Alamar blue assay. Additionally, primary DEF responded to all mycotoxins tested using the alamar blue assay, while HepG2 was less sensitive, especially at short exposure times. The estimated IC50 for aflatoxin B1, fumonisins B1, and deoxynivalenol in DEF after 72h incubation were 3.69, 4.19, and 1.26 $\mu\text{g/ml}$, respectively. Results from the current study suggest that primary DEF are more sensitive to cytotoxins and mycotoxins compared to HepG2, and thus may have great potential as an effective tool for cytotoxicity assessment. The question remains whether in vitro IC50 values can accurately predict in vivo toxicity, however, the current study accentuates the need for further attention to identify sensitive cell models for in vitro cytotoxicity screening and subsequent exploration of species-specific prediction models for in vivo toxicity.

Keywords: Cytotoxicity assay, primary duck embryonic fibroblast, HepG2, Alamar blue, MTT, mycotoxin

S4-0008 Effects of silver nanoparticles (AgNPs) on quantity and quality of sperm in Japanese quail

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The present study has examined the effects of silver nanoparticles (AgNPs) on quantity and quality characteristics of sperm in Japanese quail. The sperm was diluted in medium containing 0, 0.75, 0.125 and 0.250 ppm of silver nanoparticle. Sperm viability, Membrane integrity, and acrosomal integrity were assessed using the eosin-nigrosin test, a hypo-osmotic swelling test and a Formalin-citrate solution, respectively. Also measured motility and progressive motility and sperm concentration. The results indicated that there was no significant variation on sperm concentration compared to control group ($P > 0.05$). Addition of AgNPs in 0.125 and 0.250 ppm levels to quail semen significantly decreased ($P < 0.05$) motility and progressive motility. Also Viability was significantly reduced in 0.125 and 0.250 ppm AgNPs. The data presented that the acrosome integrity was significantly decreased at 0.125 AgNPs ($p < 0.05$). The percentage of spermatozoa with an intact membrane ($p < 0.05$) were significantly decreased in treatments containing 0.125 and 0.250 ppm AgNPs in comparison to control group. This study suggests that AgNPs may have cytotoxic effect on spermatozoa by affecting sperm functionality and causing high amount of sperm mortality.

Keywords: nanotechnology, silver nanoparticles, sperm, Japanese quail

S4-0009 Study on high hatchability and efficiency method for egg windowing

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In order to search for the main influencing factors, optimize the methods and enhance the hatchability for the windowed eggs, several experiments were made based on the former patent of chicken egg equatorial plane windowing methods, such as the sealing techniques, air chamber recovering, laying position after sealed, turning angle and relative humidity while incubated, as well as the DMEM injection dose into the sub-germinal cavity of the blastoderm. The result showed that, the highest hatchability was 27.5% for the group of the Straw Powder (SP) with instant glue (IG) among different seal technique groups. There was highly positive correlation between air chamber rate and hatchability, the higher the air chamber rate was, the higher the hatchability happened, but the average hatchability of air chamber recovered group was low (10.6%). Both control and windowed groups, for the hatchability of turning angle, group 90° was better than that of group 50°. For the hatchability of humidity, the group 70% was better than that of group 65%. The highest hatchability was raised to 68.8% when the modified techniques were used, such as the IG was dropped firstly, the SP was sprinkled secondly, and then the egg was laid down by sharp end immediately after sealed. The egg windowing and injecting had extremely significant influence on the hatchability ($P < 0.01$). The hatchability was significantly declined when the injection dose was increased ($P < 0.01$), and 1uL DMEM got the highest hatchability (48.4%), and there was extremely significant difference with that of other groups (3, 5, and 10 uL) ($P < 0.01$). So, the modified egg windowing techniques might be broadly used in the fields of avian transgenesis, genetic resources preservation and embryo development model for human medicine.

Keywords: chicken, egg windowing, air chamber, blastoderm, hatching condition, hatchability

S4-0010 Effect of in ovo synbiotic injection on productive traits, meat quality and caecal microbiota in broiler chicken

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The effects of two synbiotics, in ovo injected, on productive traits, meat quality and selected intestinal microbiota population of broiler chickens were evaluated. On the 12th day of incubation, 5,850 Cobb500 eggs were randomly distributed into 3 groups and were in ovo injected with: S1 - *Lactobacillus salivarius* with Bi2tos containing galactooligosaccharides, S2 - *Lactobacillus plantarum* combined with Raffinose Family Oligosaccharides isolated from lupin seeds and control (C) - 0.9% solution of NaCl. Dosages of synbiotics equalled to 105 bacteria cfu/egg and 2mg/egg of prebiotic. 2,040 cocks were grown to 42 days in pens of 75 birds (8 replications per treatment). Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), on a pen basis, and mortality were calculated. At 42 d of age, 15 randomly chosen birds per group were individually weighed and slaughtered. The hatchability, BWG, FI and FCR were not affected by in ovo treatment ($P > 0.05$) and averaged 91%, 3,087g, 4,923g and 1.59g/g respectively. Mortality (%) was lower in S1 (0.8) and S2 (1.2) groups compared to C group (1.8). C group had the highest number of the total bacteria in caecal digesta ($P=0.0001$). Both synbiotics lowered *Bacteroides* - *Prevotella* cluster and S1 group showed the highest counts of *Lactobacillus* spp. ($P=0.0001$). Both synbiotics had no effect ($P>0.05$) on growth and slaughter traits. Meat from S2 group displayed a significantly lower ($P > 0.05$) fat content (1.18g/100g) and a higher S/A proportion (1.76, $P > 0.05$) compared to meat from C group (1.51g/100g, 1.6). The research leading to these results has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 311794. Co-financed from funds for science allocated to an international project in the years 2013-2017.

Keywords: in ovo technology, synbiotics, chicken production

S4-0011 Transcriptome analysis of genes in the protein biosynthesis and ubiquitin-proteasome pathways in meat-type chickens under heat stress

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Growth is significantly reduced in poultry under heat stress and several strategies have been suggested to remedy the effects of heat stress with varying success. Molecular mechanisms that underlie protein biosynthesis of poultry under heat stress would allow for strategies to mitigate the effects of heat stress. We investigated the immediate and long term transcriptomics changes in chickens under heat stress. Forty-eight Cobb500 male birds were divided into two groups and raised under either constant 25C or 35C from 14-26 days of age in individual cages and fed ad libitum on a diet containing 21% CP and 3100kcal ME/kg. Five birds per treatment at 1 and 12 days after heat treatment were euthanized and the Pectoralis (P) major was sampled for gene expression analysis. mRNA expression of key genes in the avian target of rapamycin (avTOR) and the ubiquitin-proteasome pathways representing protein synthesis and breakdown, respectively were studied. Feed intake and growth were reduced in the heat stressed birds compared to the controls, whereas FCR and rectal temperature were similar among the two groups. The P. major mRNA expression analyzed by the delta delta Ct method indicated that, after a day of heat stress, avTOR and EIF4E expressions as well as UBE21, UBE3A, PSMC1, PSMD1 and FBXO32 were reduced in the stressed birds compared to controls suggesting that both protein synthesis and protein breakdown were significantly reduced. However, after 12 days of heat stress, there was an increased mRNA expression of UBE21, UBE3A, PSMC1, PSMD1 and FBXO32 compared to the controls suggesting a significant increase in protein ubiquitination and degradation. The results from this study suggest that the initial molecular response of birds to heat stress is to reduce both protein synthesis and degradation to maintain body weight. However, if the heat stress persists, protein breakdown is elevated relative to their control counterpart reflecting the differences in growth.

Keywords: heat stress, protein biosynthesis, ubiquitin-proteasome, transcriptomics

S4- 0012 Anti-leptin receptor antibodies strengthen leptin biofunction in growing chickens

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Anti-leptin receptor antibodies were utilized to study the regulatory biofunctions of de novo leptin in growing chickens. Both poly-clonal and mono-clonal anti-leptin receptor (lepR) antibodies (10 mg) to extracellular domains of chicken leptin receptor were administered i.m. to 30-d-old Gushi breed pullets (n=15) of similar body weight, in contrast to the physiological saline treated controls. Feed intake was measured till 6 h post administration, and the birds were slaughtered for collection of hypothalamus, pituitary gland, liver, abdominal fat, and breast muscle tissues. Administration of both kinds of antibodies increased food intake in the 6 h of treatment, and reduced plasma concentrations of glucose, triglyceride, high-density lipoprotein, and low-density lipoprotein. Paralleled by increase in feed intake, mRNA gene expression levels of agouti-related peptide gene and neuropeptide Y were up regulated, but of proopiomelanocortin, melanocortin 4 receptor and lepR down-regulated in antibodies treated chicken hypothalamic. The antibody treatment also down regulated the gene expression levels of lepR, fasn, but up regulated AMP-activated protein kinase, acetyl CoA carboxylase-2, and uncoupling protein 3 in liver, abdominal fat, and breast muscle ($P<0.05$). These results demonstrate that antibodies to membrane proximal lepR extracellular domain enhanced lepR signal transduction, which increase food intake and stimulates metabolism and reduces fat synthesis in chickens.

Keywords: growing chicken, anti-leptin receptor antibodies, feed intake, blood metabolite concentrations, gene expressions

S4-0013 Reproductive axis gene regulation of photostimulation and photorefractoriness of Yangzhou goose ganders

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Yangzhou goose is a long day breeder that has been increasingly produced in eastern China provinces. This study therefore was carried out to investigate the regulatory mechanisms by artificial photoperiodic programs on reproductive activities of Yangzhou goose ganders. A flock of Yangzhou goose replacement fowls aged 6 months were divided into 2 groups of 300 ganders. Both flocks were initially exposed to a short daily photoperiod of 8 h (8L: 16D) for either 2 (Group A) or 3 (Group B) months, and then both were switched, respectively, to a longer 12 h daily photoperiod (12L: 12D), for a duration of 8 months. Increasing daily photoperiod from 8 h to 12 h rapidly stimulated testis expansion, and increased plasma testosterone concentrations, in both groups of ganders, which both reached peak levels at 2 months after switching long 12 h daily photoperiod. This stimulation of reproductive activities was associated decreases of GnRH and GnIH gene mRNA expression levels in the hypothalamus, with the decrease of GnIH being more pronounced than GnRH. In the pituitary gland, expressions of FSH and LH mRNA all abruptly increased to high levels under long 12 h photoperiod. After the peak level, plasma testosterone concentrations continued to decrease to low values in the later 6 months of the experiment. This occurrence of photorefractoriness was associated with an increased GnIH genes expression level by over 250-folds, and then PRL and TSH in pituitary gland. The photostimulation reproductive activities in Yangzhou goose ganders is regulated by a lowering of GnIH mRNA expression in the hypothalamus, while the photorefractoriness was mediated by an increase in GnIH, and subsequently VIP and TRH gene mRNA expressions in the hypothalamus. Changes in gene expression levels of these neuronal hormones directed gene expressions and secretions of LH/FSH, PRL and TSH in the pituitary gland, which dictated photoperiodic regulation of waxing and waning of the testes.

Keywords: Yangzhou goose gander, reproductive activities, photoperiod, gene mRNA expressions

S4- 0014 Efficient enrichment of modified chicken PGCs using CRISPR/Cas9

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Chicken primordial germ cells (PGCs) have been used to manipulate the germline for the generation of transgenic chickens. Unfortunately, a low modification rate has limited their use and applicability in the field. Recently, clustered regularly interspaced short palindromic repeat (CRISPR)-associated (Cas) 9 technology has revolutionized the precise genome editing field given its simple-design, low-cost and high-efficiency. The CRISPR/Cas9 system originated from the prokaryotic adaptive immune system and contains a short guide RNA (gRNA) complementary to the unique target sequence and a Cas9 endonuclease that cleaves the target gene upon recognition of the protospacer- adjacent motif (PAM, 5' NGG 3') immediately downstream of the target sequence. The aim of our study was to modify PGCs in proof-of-principle experiments for the generation of transgenic chickens. We chose to interrupt the interleukin 6 (IL6) gene in chicken PGCs using a co-targeting CRISPR/Cas9 system given its prominent role in avian influenza virus infection. Flow cytometric sorting was used to enrich our target PGC population. We demonstrated that 55.7% of enriched PGCs contained the IL6 gene deletion. Moreover our modified PGCs were able to migrate to gonads when injected into recipient chicken embryos. PCR analysis showed that 9.8% of total gonad PGCs isolated from recipient embryos contained the IL6 gene deletion. This novel strategy allows efficacious enrichment of manipulated chicken PGCs and lays a foundation for the future generation of knockout chicken lines.

Keywords: PGCs, CRISPR/Cas9, Co-target, IL6

S4- 0015 Molecular mechanisms of perturbed goose ovarian follicle development by environmental bacterial endotoxine Lipopolysaccharide

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The aim of this study was to investigate the molecular mechanisms of impairment of reproductive performances by environmental endotoxin lipopolysaccharide (LPS). Yangzhou goose at peak of lay were administered with a bolus of 1.5 mg/kg BW LPS (serotype O55:B5, Sigma, St Louis, USA), and were slaughtered approximately 8 h after oviposition. The ovary, plasma, and the theca and granulosa layers of all hierarchical follicles were collected at 0, 3, 6, 12, 24 and 36h after the administration of LPS (n = 5). The morphology and color of ovarian follicles were not changed at 0, 3, 6 and 12 h after administration of LPS, however, some hierarchical follicles became irregular ellipse/circle and deep yellowish (IDF) at 24 h. The circulating E2 concentration was significantly decreased alone the time after LPS administration. Plasma P4 concentration decreased during the 6 to 36 h subsequent to LPS treatment. mRNA expression of CYP11A1, associated with P4 biosynthesis, increased initially at 3 h, then gradually declined to the lowest level during the 6 to 24 h in follicular granulosa layers. mRNA expression of STAR, also associated with P4 biosynthesis, increased at 6 and 12 h, but level declined to the lowest value at 24 and 36 h in follicular granulosa layers. The mRNA expression of CYP17A1 and CYP19A1, associated with E2 biosynthesis, was significantly decreased during the 0 to 24h period in follicular theca layers. Except for CYP11A1, STAR, CYP17A1 and CYP19A1 mRNA expression could be detected in IDF, moreover, compared with those at 24 h, CYP17A1 and CYP19A1 mRNA expression increased at 36 h after LPS treatment. In conclusion, these results suggest a potential mechanism by which gram-negative bacterium, such as *E. coli* and *Salmonella* Enteritidis, decreased egg production may involve responses to disrupted ovarian steroid hormones biosynthesis.

Keywords: goose, lipopolysaccharide, steroid hormones, hierarchical follicles

S4-0016 The effects of different levels of vitamin E in semen extender on rooster sperm quality during chilled storage

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Background: Semen storage in domestic birds has been studied extensively in the past fifty years. However, it induces partially irreversible damages to sperm. These damages can deteriorate anatomical, and structural organs in sperm. One the other hand, Oxidative damage to sperm resulting from reactive oxygen species generated by the cellular components of semen is one of the main causes for the decline in motility and fertility of sperm during the cold storage. **Objective:** Vitamin E is considered to be the main component of the antioxidant system of spermatozoa. Therefore, the present study was conducted to determine the effects of vitamin E in the modified Beltsville extender on some rooster semen quality parameters at 4 ° C. **Materials & Methods:** Semen was collected from ten With Leghorn roosters twice a week. Ejaculates with greater than 80% forward spermatozoa motility were pooled and after dilution semen (with modified Beltsville extender) was enriched with 0 (control), or containing 5 (E5), 10 (E10) and 20 (E20) µg/mL vitamin E. Forward spermatozoa progressive motility and viability, as well as sperm abnormality were evaluated at 0, 24, and 48 h of storage. Achieved data were analyzed using Repeated Measure ANOVA. **Results:** The results showed that E5 and E10 extenders resulted in higher forward motility and sperm viability ($P < 0.05$) than the other extenders at 24 and 48 h of storage. The E20 extender showed deleterious effects on sperm motility and viability and also induced sperm abnormality in compared with other extenders. **Conclusion:** The results of the present study demonstrated that E5 and E10 can improve the function of rooster sperm during the cold storage.

Keywords: Vitamin E, rooster, Semen, chilled storage, progressive motility

S4- 0017 Effects of Nano Selenium on some parameters of rooster semen during chilled storage

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Background: The practice of artificial insemination is widely utilized in poultry; and this requires a broad use of semen storage techniques to prevent the reduction of fertilizing ability of stored semen. It has been suggested that antioxidant's addition to semen can improve sperm motility and viability during cold storage. Selenium is a constituent of selenoproteins such as glutathione peroxidase (GSH- Px) that protect against oxidative damage to spermatozoa. Studies on Nano Selenium (Nano-Se) confirm its efficacy on inducing selenoproteins with lower toxicity (vs. selenium) and acceptable bioavailability. **Objectives:** The present study was designed to evaluate the effect of Nano Selenium on the quality of rooster semen stored at 4 ° C. **Material & Methods:** Semen was collected from ten With Leghorn roosters twice a week. Ejaculates with greater than 80% forward spermatozoa motility were pooled and after dilution semen (with modified Beltsville extender) was enriched with 0 (control), or containing 0.5%, 1% and 2% Nano-Se. Spermatozoa progressive motility and viability, as well as sperm abnormality were evaluated at 0, 24, and 48 h of storage. Achieved data were analyzed using Repeated Measure ANOVA. **Results:** Motility was 78.76, 80.74, and 67.56% (± 1.75) at 24 h and 62.83, 63.32 and 58.03% (± 1.90) at 48 h in 0.5% Nano-Se, 1% Nano-Se and control, respectively ($P < 0.05$). Furthermore, Both 0.5% Nano-Se and 1% Nano-Se had greater viability at 24 (74.51 and 79.45% ± 1.87) and 48 h (65.70 and 69.31% ± 2.02) in contrast to control (67.03% ± 1.87 and 60.06% ± 2.02 , respectively at 24 and 48 h) ($P < 0.05$). Sperm abnormality was higher in 2% Nano-Se than 0.5% Nano-Se and 1% Nano-Se ($P < 0.05$) but was similar to control group at 24 and 48 h of storage. **Conclusion:** enrichment of rooster semen with small doses of Nano-Se had beneficial effects on the semen quality during cold storage.

Keywords: nano selenium, semen, rooster, chilled storage, progressive motility

S4- 0018 Chicken germline chimera production: optimization of gonadal primordial germ cells isolation and selection of optimal donor/recipient combination

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The aim of this study was to develop the method of chicken germline chimeras production by the optimizing of gonadal primordial germ cells (gPGC) isolation, and the selection of gPGC of donor/recipient optimal combination. In the first experiment 6-day-old donor embryos (n 72) were randomly divided into three groups, and their gonads were: A- mechanically fragmented; B- partially digested (0.25% trypsin- EDTA solution); C- untouched; placed in 0.5 ml PBS[-] and incubated for 1 hour in 37.8°C. The survival rate of gPGC stained with 0.4% trypan blue were determined. In the second experiment, Ross 308 (R) and Green legged Partridgelike (GP) embryos were used both as donor and recipient of gPGC. Cells isolated according to the method optimized in the first experiment, stained with PKH26 fluorochrome, and 1000 of labeled gPGC were injected into dorsal aorta of 3.5-day-old recipients. Manipulated embryos were incubated until stage 28-29 (HH), then both gonads were collected and fluorescence was detected under fluorescent microscope in wavelength range of 551- 567 nm. The percent of embryos with labeled exogenous gPGC in gonads was calculated. The highest number of morphologically normal gPGC (114 000; $P < 0.05$) that did not form aggregates was obtained in group B. The highest percent (54.5; $P < 0.01$) of embryos with exogenous, PKH26 labeled gPGC was present in the group R/GP, as a donor/recipient of cells, respectively.

Keywords: primordial germ cells, chicken gonads, chicken chimera, gonadal germ cells

S4- 0019 Exogenous melatonin improved performance of laying hens after egg peak

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Introduction: A large number of studies show inhibitory effects of melatonin on poultry reproduction, however melatonin can also reduce the stress of laying hens as antioxidant. This is the first study of melatonin for delaying the decrease of laying rate and achieved remarkable results. **Materials and methods:** The melatonin implants were made by Chinese Academy of Agricultural . 120 hens of 300days old and were selected and were divided into four groups, and treated with 5mg, 10mg, 20mg and 0mg of melatonin implants respectively. 60 hens of 550days were treated like 300days. Divide 240 hens of 360 (and 470) days in four and treat them with 1mg, 2mg, 3mg, and 0mg of melatonin respectively. **Results:** When laying hens are implanted at 300 days old , the egg laying rate increment after the implantation of the group implanted with 1 grain is higher than the control on average 4.63%. The egg laying rate increment after the implantation of the group implanted with 10mg is higher than the control on average 8.38% in the age of 360days . The egg laying rate increment after the implantation of the group implanted with 1 grain is higher than the control on average 4.93% in the age of 470days. The egg laying rate increment after the implantation of the group implanted with 10mg is higher than the control on average 8.01% in the age of 550days. Implantation at 300 days with 5mg in the short term can inhibit the drop of egg laying rate. Implantation at 360 and 550 days with 20mg also show benefit effect on egg laying rate 1 month after implantation. Implantation with 1 grain at 360 days of age can improve the serum estradiol content and significantly increased the expression of MT2. however, significantly reduced the expression of GnIHR It means that melatonin may improve egg laying rate by increasing serum estradiol and decreasing ovarian GnIHR, and MT2 may be involved in. **Conclusion:** Implantation with 10mg melatonin could improve performance of laying hens after egg peak.

Keywords: chicken, melatonin, egg peak, egg laying rate, MT2.

S4- 0020 Response of the Toll-like receptor signaling pathway to avian infectious bronchitis virus infection in chicks

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Avian infectious bronchitis virus (IBV) is a Gamma-coronavirus in the family Coronaviridae and causes highly contagious respiratory disease in chickens. Innate immunity plays significant roles in host defense against IBV. Here, we explored the interaction between IBV and the host innate immune system. 2-week-old specific pathogen-free chicks were oculonasally with IBV M41. At 1, 3, 5, 8, 11, 14, 21, and 28 days postinoculation (dpi), three birds in each group were bled before euthanasia and necropsy. The clinical signs and histopathologic lesions in IBV M41 infected chickens were observed. The viral load and mRNA dynamic of TLR signal pathway molecules and innate immune cytokines in trachea and kidney tissues were detected by the real-time quantitative PCR, and the concentration of these cytokines were measured using the ELISA method. Severe histopathological lesions were observed in the trachea at 3 – 5 dpi and in the kidney at 8 dpi, with heavy viral loads at 1 – 11 and 1 – 28 dpi, respectively, while the viral load in kidney was lower than that of the trachea. The expression of mRNAs encoding TLR3 and TLR7 were upregulated at 3 – 8 dpi, and TRIF was upregulated at 21 dpi in the trachea and kidney. MyD88 was upregulated in the trachea during early infection. Tumor necrosis factor receptor-associated factor (TRAF) 3 and TRAF6 were upregulated expression in both tissues. Moreover, TBK1, inhibitor of kappaB kinase (IKK) ϵ , IKK α , IKK β , IRF7, NF- κ B, IFN- α , IFN- β , IL-1 β , IL-6, IL-8, IL-10, IL-12 and MIP-1 β were significantly upregulated in the trachea and down-regulated in the kidney. The protein levels of these cytokines were higher than those of the control group in trachea, but were discrepant in kidney. These results suggested that the TLR signaling pathway and innate immune cytokines were induced after IBV infection. Additionally, consistent responses to IBV infection were observed during early infection, with differential and complicated responses in the kidney.

Keywords: infectious bronchitis virus, TLRs signal pathway, innate immune tokines, pathological changes

S4- 0021 Mitochondrial quantification and expression of genes associated with protoporphyrin IX synthesis in the shell gland of laying hens in response to nicarbazin

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Mitochondrial quantification was performed in the shell gland of laying hens treated with nicarbazin using a quantitative PCR assay. Furthermore, expression of 6 genes associated with protoporphyrin IX (PP IX) synthesis and/or deposition was examined in order to assess whether the treatments can lead to differential expression of the genes. The results showed that the mitochondrial concentrations per cell were significantly lower in the nicarbazin-fed group at 15 hr post-oviposition time. In the control group, the expression of ALAS1, FECH and SLC25A38 were significantly different at different stages post-oviposition. In the nicarbazin group, the expressions of all genes except CPOX were significantly different at different stages post-oviposition. The PP IX content per gram of shell gland tissue was significantly lower ($P < 0.05$) in the nicarbazin-fed birds compared with the control group. The amount of PP IX in whole eggshell decreased with day effect in the nicarbazin group, in a linear pattern, but it remained constant in the control group. However, egg weight and shell thickness were not significantly different between the control and nicarbazin-fed groups. It can be concluded that the mitochondrial concentrations in the shell gland were altered by nicarbazin only at 15 hr post oviposition, and the expression of some genes associated with PP IX synthesis and/or deposition were affected by post-oviposition time rather than by nicarbazin. The complete discolouration of brown eggshell pigment in the nicarbazin-fed groups may highlight the fact that there are some other genes and/or regulators involved in PP IX synthesis and/or deposition that need to be investigated further.

Keywords: eggshell, protoporphyrin, mRNA, drug, oviposition time

S4-0022 The effects of different levels of Nano Selenium as a antioxidant on rooster's post-thawed sperm quality

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Semen cryopreservation is very important for the ex situ management of genetic diversity in birds but it is rarely used. As previously reported, sperm motility and viability are the most commonly affected characteristics during cryopreservation and thus are the main cause for the reduced fertility after freezing/thawing processes. It has been suggested that antioxidant's addition to semen before cryopreservation can improve sperm motility and viability during freezing/thawing processes. Selenium is a constituent of selenoproteins such as glutathione peroxidase (GSH-Px) that protect against oxidative damage to spermatozoa. Studies on Nano Selenium (Nano-Se) confirm its efficacy on inducing selenoproteins with lower toxicity (vs. selenium) and acceptable bioavailability. The present study was conducted to determine the effects of different levels of Nano selenium on some post-thaw rooster semen quality parameters. Semen samples from 10 sexually-mature White Leghorn roosters were collected and pooled, divided into four equal parts and diluted with Beltsville extender containing with no Nano-Se (control), or containing 0.5%, 1% and 2% Nano-Se. The sperm motility and progressive motility after thawing was assessed by CASA. Also sperm viability were assessed by Eosin-Nigrosin staining. The data were analyzed by the GLM procedure of SAS 9.1. Using Nano-Se at 0.5% and 1% significantly increased sperm motility (67.5 and 71.25%, respectively; $P \leq 0.05$), progressive motility (15.48 and 16.23%, respectively; $P \leq 0.05$) and viability (70.46 and 72.52%, respectively; $P \leq 0.05$) in compared with control. But 2% Nano-Se had deleterious effects on rooster semen. Results of this study revealed that addition of 0.5% and 1% Nano-Se to the extender for freezing of rooster semen can improve significantly the function of post-thawed rooster spermatozoa.

Keywords: sperm motility, Nano Selenium, antioxidant, cryopreservation, rooster.

S4- 0023 Effect of different inseminated sperm number on early embryonic mortality in chicken

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Polyspermy is a known phenomenon in avian species, although only one fuses with the female pronucleus and the others penetrate the perivitelline layer of the vitellus. The exact role and the mechanism of the entered extra spermatozoa are still unclear. Earlier papers reported that too low or too high sperm concentration may cause early embryonic mortality (EEM), thus the normal embryonic development needs an appropriate number of penetrated spermatozoa. The aim of this study was to clarify the relationship between the inseminated sperm number and EEM as well as the effect of high sperm concentration to the fertility. During 11 weeks 20-20 Tetra SL hens were inseminated with extremely low (EL), high (H) (compare to normal protocol) and extremely high (EH) sperm concentration, that is 2 x 1 million, 2 x 300 million and 3 x 1000 million spermatozoa/hen/week, respectively. The efficiency of insemination was checked by in vitro perivitelline sperm penetration assay (PSPA) on freshly laid eggs. EEM was determined following the candling on the 7th day of incubation. In PSPA significantly lower penetration holes were detected in EL group while the EEM was significantly higher – more than 25% and, where the very early, oviducal embryonic mortality was for the most part. In H and EH groups the inseminated sperm numbers did not influence either the ratio of EEM (1%), or the fertility level (94% and 97%) even though the difference between the sperm concentrations was very big in the groups. It confirmed that the vagina, the sperm storage tubules as well as the oviduct have a complex common regulatory mechanism, which preserves the ovum from the adversely high dose of sperm. It was proved that increasing the inseminated sperm concentration to over 300 million is unnecessary and uneconomical. The low number of spermatozoa causes embryonic death mostly at the earliest stage of development when the embryo is still within the oviduct.

Keywords: artificial insemination, chicken, polyspermy, early embryonic mortality

S4- 0024 Knocking down the myostatin gene to determine growth in chicken

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Myostatin is a member of transforming growth factor-beta superfamily and acts as negative regulator of muscular growth in poultry. To minimize the activity of myostatin, knocking down the gene is an efficient approach to augment muscular growth in chicken. A total of 5 shRNA molecules were designed from coding sequence of mRNA of chicken MSTN gene. The shRNA molecules were cloned in pBLOCK-ITm U6-DEST vector and transferred to the chicken through in ovo injection on day4 of embryonic stage (Hamburger-Hamilton stage 23). A total of 56 eggs were manipulated from which 36 chicks were hatched where fertility, hatchability on fertile and total egg set basis were 84, 76.1 and 64.2%, respectively. The mRNA expression of myostatin gene in knock-down chicken was significantly ($P<0.01$) lower than that of control birds where knock down% of myostatin gene varied from 7 to 22%. However, the effect of the shRNA molecules on post-hatch body weight was found to be significantly ($P<0.05$) higher at 4 and 6 weeks of age as compared to control birds. At 6 weeks of age, molecule 3 showed the highest effect by increasing body weight with 26.9 % while molecule 1 had 22 % higher body weight than control group. The molecule 2, 4 and 5, respectively showed 23.4, 26.4 and 24.4 % higher body weight at 6 weeks of age. It is concluded that knocking down of the myostatin gene may increase juvenile growth in chicken.

Keywords: chicken, growth, knock down, myostatin

S4- 0025 Germinal disc region: an appropriate source for obtaining maternal DNA

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Egg compositions that few cells are present in the environment rich in proteins and lipids pose a challenge on DNA isolation. Previously, eggshell, yolk and whole egg liquid (WEL) have been used as DNA sources. However, poor quality and high rate of PCR failure indicated that an improvement in egg-derived DNA isolation remains necessary. Here, germinal disc region (GDR) was used as DNA source. DNA yield and PCR efficiency in the GDR protocols were compared to those in previous eggshell and WEL protocols. Eggshell, WEL and GDR DNA could not be evaluated spectrophotometrically, nor could they be visualized on the agarose gel, indicating that DNA yield was extremely low whichever egg fractions were used. Although DNA yield fell short of our expectation in the GDR protocol, PCR efficiency in GDR DNA as template achieved a significant improvement with ratios of PCR success increased to 73 % for amplification of short fragments (< 300 bp), 11-43 % for long fragments (> 1 kb), significantly higher than ones in the cases of WEL and eggshell DNA as template ($P < 0.05$). After whole genome amplification (WGA) was introduced, GDR DNA yield was significantly increased. Not only did the post-WGA GDR DNA further improved PCR efficiency, but it can meet the need of next-generation sequencing. Our study indicated that eggshell and WEL were not the optimal DNA source. GDR DNA extraction followed by WGA not only improve PCR efficiency greatly, but technologically overcome the limit in DNA yield from single unfertilized egg, thus providing a feasible pipeline to obtain sufficient egg-derived DNA for subsequent variant analysis.

Keywords: egg, DNA extraction, germinal disc region, whole genome amplification, next-generation sequencing

S4-0026 Association of seminal constituents and plasma testosterone concentration with certain sperm quality attributes in broiler breeders

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The ultimate quality of sperm in ejaculated semen is the reflection of its internal environment which warrants further investigation. The present work is an attempt to investigate the hormonal, seminal trace elemental and metabolic constituents involvement in defining the sperm quality in broiler breeders. Fifty adult (35 weeks) healthy broiler breeders (n=50) of Coloured Synthetic Male Line were chosen randomly and neat semen was collected on individual basis. Sperm motility, concentration, percent livability and functional integrity of sperm plasma membrane were assessed in neat semen as per standard protocols. Trace minerals (Zinc, Copper, Iron and Cobalt) and metabolic constituents (Calcium, Phosphorus, Total Protein, Cholesterol, Triglycerides, Alanine Aminotransferase-ALT and Aspartate Aminotransferase-AST) were estimated in seminal plasma by employing atomic absorption spectrophotometry and standard kits respectively. ELISA based plasma testosterone profile was investigated to arrive at hormonal correlation. Results generated from regression analysis indicated that sperm motility ($R^2 = 0.93$) and livability ($R^2 = 0.89$) are positively influenced by higher concentrations of Zn, Cu, Co, Ca, ALT, testosterone and negatively affected by Fe, P, protein, cholesterol, triglycerides and AST levels. Similarly, plasma membrane integrity ($R^2 = 0.81$) was in positive correlation with Zn, Cu, Co, Ca, P, triglycerides, ALT and negatively with Fe, protein, cholesterol, AST and testosterone levels. The total sperm concentration ($R^2 = 0.70$) was negatively affected by Fe, triglycerides, ALT and AST enzymes. In conclusion, the sperm quality can be majorly influenced by the varying concentrations of metabolic and trace elemental make up of seminal plasma and circulating levels of testosterone. These findings will allow us to gain new insights on sperm motility and its predicted fate in oviduct. Further, breeder dietary requirements can be redefined to meet quality sperm requirements.

Keywords: broiler breeders, trace elements, metabolic constituents, testosterone, sperm quality

S4-0027 A simple methods for isolation of circulating primordial germ cells from avian species

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Primordial germ cells (PGCs) are the precursors of gametes in sexually reproducing animals. Therefore the cells are considered to be crucial in development and extensively studied in the field of reproductive biology. In avian species, PGCs migrate to the gonadal ridge through bloodstream in the early embryonic stages. This unique characteristics provide benefits for the study in restoration of endangered avian species, because it is able accessibility to isolate PGCs from the embryonic blood vessels without sacrificing embryos. The previous methods for blood PGC (bPGC) isolation needed specific antibodies and have shown low yield rate. In this study, we isolated bPGCs from chicken embryos by differences of cell size between blood cells and PGCs using the microporous membranes. The strained cells were positive for the chicken PGC markers and expressed PGC- specific genes. Moreover, these cells have also shown migrational activity. In addition, this system was confirmed in other avian species. Our results demonstrate that the avian bPGCs can be simply isolated by cell size without any specific markers and it will be applicable to restore endangered avian species.

Keywords: chicken, endangered avian species, primordial germ cells, PGC isolation

S4-0028 Epithelial progenitor (stem-like) gene expression signatures in oviduct of laying hen (*Gallus gallus domesticus*) and quail (*Coturnix japonicus*)

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We aimed to identify a region in oviduct of laying hen and quail where putative progenitor (stem-like) cells reside. Epithelial progenitors are required to maintain proliferation in vitro and to develop an oviduct epithelial cell line (OEC). Identification of stem-like cells with epithelial genetic markers is necessary to select a source tissue for establishment of a stable OEC. Oviducts were isolated from laying hens (n=6, age 40 wks) and quails (n=5, age 15 wks). RNA isolation was performed with Trizol and the primers were designed based on chicken cDNA sequences; 3 gene panels were selected as markers for distinct cell types: 1) oviduct - ESR1, OVAL and OVM; 2) epithelial - KRT5, KRT14, OCLN and 3) progenitor - Msi1, SOX9, NANOG, OCT4, CD44. Relative gene expression was conducted with RT-qPCR in 3 oviduct regions: infundibulum neck (INF), distal magnum (DM) and proximal magnum (PM) in hen and quail. Pectoral muscle was used as a calibrator. Fold induction was calculated with ddCt method using ubiquitin C and β -actin as reference genes. Oviduct markers OVAL and OVM were down-regulated in INF and gradually up-regulated in DM and PM. ESR1 was up-regulated in all regions, with increased expression in PM and DM in both hen ($P<0.05$) and quail ($P<0.01$). The strongest expression of epithelial markers: KRT5, KRT14 in both species ($P<0.01$) and OCLN in hen ($P<0.01$) was found in INF. Progenitor markers: Msi1, SOX9, CD44 ($P<0.01$), NANOG ($P<0.05$) in both species and OCT4/cPOUV ($P<0.01$) in quail were highly expressed in INF and decreased in proximal parts of oviduct. Expression profile of epithelial progenitor markers was reflected in our in vitro studies (higher proliferation rate of OEC from distal oviduct as compared to OEC of proximal regions). In conclusion, hereby we validated INF as a source of stem-like cells for OEC establishment in hen and quail, using a panel of gene expression signatures. Grant: NCN/2011/03/N/NZ9/03814.

Keywords: oviduct, laying hen, quail, epithelial progenitor cell marker, gene expression

S4-0029 Development of a plasmid-based methods for expression and antisense gene knockdown in *Riemerella anatipestifer* and application

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Riemerella anatipestifer is a duck pathogen that causes serious economic loss to duck industry in worldwide. However, a study of the pathogenic mechanism of *Riemerella anatipestifer* is less, since the genetic tools have not been completely established. Here, we first constructed three *Escherichia-Riemerella anatipestifer* shuttle vectors that contained three different native *Riemerella anatipestifer* promoters, respectively. Shuttle plasmids were introduced into *Riemerella anatipestifer* ATCC11845 and *Riemerella anatipestifer* CH-1 by conjugation using the origin of transfer (oriT) of RP4 at an efficiency of 5×10^{-5} and 10^{-6} antibiotic-resistant transconjugants per recipient cell, respectively. Taking advantage of these shuttle plasmids, the tonB1 gene was inserted into these plasmids respectively, and introduced into *Riemerella anatipestifer* ATCC11845 Δ tonB1, demonstrating that this system can be used for the study of gene complementation. Based on a high level expression shuttle vector pLMF03, a method used for knockdown was established. Knockdown of TonB family protein in *Riemerella anatipestifer* ATCC11845 decreased the growth ability in TSB medium and hemin utilization. All these genetic tools pave the way for gene function study of *Riemerella anatipestifer* and may have a wide application in many other members of the Flavobacteriaceae.

Keywords: *Riemerella anatipestifer*, genetic tools, knockdown, hemin utilization

S4-0030 Protective small heat shock protein in the chicken blastoderm

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Small heat shock proteins (sHSPs) ranging in size from 12 to 30 kDa and containing α -crystalline domain have been proposed to have functions in cellular proliferation, differentiation and first line of defense against to physiological and environmental stresses in an ATP-independent manner in other species. In chicken, newly oviposited stage X (EGK) blastoderm can survive several weeks under the low temperature storage suggesting that blastodermal cells are basically tolerant of environmental stresses. However, studies of small HSPs in early embryos have yet to be investigated in chicken. Thus, we investigated expression and function of sHSPs in chicken blastoderm. We found that chicken-specific sHSP was expressed especially in blastoderm and highly upregulated during low temperature storage. Multiple alignment and phylogenetic trees showed subfamily of this sHSP were conserved in avian species. After knockdown of chicken-specific sHSP using siRNAs, pluripotency marker genes were significantly decreased. Furthermore, chicken-specific sHSP are associated with anti-apoptotic, anti-oxidant and pro-autophagy functions in blastodermal cells. Collectively, chicken-specific sHSP are important associated with protection of future embryonic cells in chicken blastoderm.

Keywords: chicken, blastoderm, small heat shock proteins

S4- 0031 Generation of transgenic chicken producing n-3 fatty acid using piggyBac transposon-mediated gene transfer in primordial germ cell

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Omega-3 (n-3) fatty acids are beneficial nutrients mainly found in fish oil, but mammals and birds cannot naturally produce the n-3 fatty acid due to lack of n-3 desaturase. At present, the n-3 enriched poultry is produced by feeding fishmeal and other n-3 fatty acid containing marine products, but this is expensive and inefficient. Here, we successfully generated n-3 desaturase expressing chicken that can produce the n-3 fatty acid using piggyBac transposon system to manipulate the chicken primordial germ cells (PGCs). We firstly constructed a piggyBac transposon vector that express chicken codon-optimized n-3 desaturase gene from *C.elegans*. Next, we validated the function of n-3 desaturase in production of n-3 fatty acids using primary chicken adipose-derived progenitor cells (ADPCs). By analysis of gas chromatography, the significant increase of n-3 fatty acids like eicosapentaenoic acid (EPA) was detected in adipogenic differentiated chADPCs that express the n-3 desaturase. Then, we established the n-3 desaturase overexpressing PGCs lines using piggyBac transposon-mediated gene transfer system. The genomic integration and expression of n-3 desaturase gene in genetically modified PGC lines was confirmed by PCR and RT-PCR, respectively. Subsequently, we produced the n-3 desaturase expressing progeny by the n-3 desaturase transgenic PGCs transferred to recipient Korean Oge. The n-3 desaturase expressing progeny was identified by PCR and sequencing. Furthermore, the transgene integration site of the progeny was identified by DNA walking analysis. This n-3 desaturase expressing chicken can be applied for production of n-3 fatty acids enriched meats and eggs in chicken.

Keywords: omega-3 fatty acid, primordial germ cell, transgenic chicken

S4-0032 Reprogramming of chicken feather follicle cells

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For the conservation of avian species, germ cell-mediated germline transmission system have been reported to be one of the most suitable system for the restoration of endangered or high value bird species. However, germ cell-specific markers and its culture systems have not been fully developed in most aves. Avian feather is one of the most easily accessible cell source in many birds and its reprogramming into pluripotent cells can be one of the alternate systems for preservation of endangered avian species. In this study, we report the generation of chicken stem cell-like cells from chicken feather follicle cells (FFCs). The FFCs reflected their characteristics by gene, protein expression and its differentiation potentials. Next, the FFCs were reprogrammed through the overexpression of pluripotency related genes. The morphology of reprogrammed cells were distinguishable from FFCs under microscopic observation. These cells were positive to Alkaline Phosphatase (AP) and Periodic acid-Schiff (PAS) staining, and gene expression profile was different from FFCs. Immunocytochemistry and RT-PCR analyses were performed to characterize the reprogrammed cells, and the results indicated that their pluripotent characteristics were maintained during culture. Embryoid body formation was detected after 6 days of culture on a non-adhesive plate. Then the cells differentiate into different types of cells. Our results showed that chicken stem cell-like cells derived from FFCs can proliferate with the pluripotent property and differentiate into several types of cells in vitro. These cells can be useful for the production of avian species from diverse cell sources and useful for conservation of avian species.

Keywords: feather follicle cells, stem cells, pluripotency, conservation, avian species

S4-0033 Action of leptin for pituitary hormone gene expression in the chick

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Leptin is an adipokine having roles in the control of food intake, reproduction and energy metabolism. The hyperleptinemia causes obesity in mammals and that results in reproductive failure especially in particularly female. In vertebrates, leptin is also known to influence on the release of pituitary hormones such as luteinizing hormone (LH), follicular-stimulating hormone (FSH), growth hormone (GH), and prolactin (PRL) directly, or indirectly through hypothalamus. However, the direct action of leptin in regulating pituitary function is not fully understood. The aim of this study was to clarify whether leptin involves in the regulation of gene expression of pituitary hormone in chicken to better understand role of the adipokine on growth and gonadal function. Pituitary gland was excised from 10-day-old male broiler chicks, and precultured in 100µL of M199 medium for 3 h with replacing the medium every hour. After pre culture, the pituitary gland was treated with 10 or 100 ng/ml recombinant mouse leptin or saline as control, and incubated at 37 °C for 16 h. At the end of treatment, total RNA was extracted from the pituitary gland. mRNA expression of pituitary hormones and SOCS3 was analyzed by real-time PCR. No significant differences were found in LH, FSH, GH mRNA expression by leptin treatment. On the other hand, PRL mRNA expression was elevated by 10 ng/ml leptin treatment, and that significantly decreased to 37% by 100ng/ml leptin treatment. Regarding mRNA expression of SOCS3 that negatively regulates signal transduction of leptin was induced by 100 ng/ml leptin treatment, but not by 10 ng/ml leptin treatment. Inhibitory action of SOCS3 on leptin signaling may cause high dose of leptin could not increase PRL gene expression, otherwise low dose did. In conclusion, the leptin does not have a great effect on the mRNA expression of gonadotropin and growth hormone in chicken pituitary gland, but it might control transactivation of PRL mRNA.

Keywords: chicken, leptin, pituitary gland, prolactin

S4-0034 Sperm storage : Ultrastructural investigation of avian spermatozoa incubated in selected fractions of uterine fluid

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Hens keep spermatozoa in their genital tract for long periods, and produce fertilized eggs for up to 3 weeks after insemination. Storage mechanisms and oviducal environment impact on sperm are largely unknown. The aim of this study was to evaluate the uterine fluid (UF) effect on semen using two different lines of hens that display a long (21 days, F+ line) or a short (10 days, F- line) period of sperm storage. UF was collected 10h after oviposition. Fresh ejaculates were used in both experiments. For the first one, spermatozoa were incubated in vitro in either PBS alone, PBS containing 25% or 50% UF, or in pure UF from both lines for 5 min, 1, 2.5, 5 and 24h at 4°C. For the second experiment, UF from both lines were depleted from proteins >3kDa (d-UF) using Vivaspin 500. Spermatozoa were incubated in vitro in PBS, d-UF, and pure UF for 5 min, 1 and 2.5h at 4°C. Sperm motility was assessed using computerized method at 41°C (HTM-IVOS II). Metabolites contained in d-UF from both lines were quantified by NMR and sperm morphology was analyzed using Atomic Force Microscopy (AFM). We observed that pure UF and d-UF improve sperm motility compared to PBS. Nevertheless the effect of d-UF was lower than pure UF. In both experiments, sperm motility was higher after incubation in UF or d-UF from F-line than F+ line. NMR analysis reveals that the concentrations of 5 metabolites were higher in d-UF from F+ than F- line ($P < 0.05$), including alanine, succinate, dimethylamide and N-acetyl groups. Moreover, AFM analysis clearly showed an alteration of head morphology of spermatozoa incubated with d-UF from the F- line. This study clearly demonstrates the major role of UF proteins >3kDa on the sperm motility. Nevertheless, the UF fraction (<3kDa) which mainly contains peptides and metabolites improves sperm motility and leads to ultrastructural modification of spermatozoa. Our findings demonstrate that the microenvironment dynamism and complexity is a key element during sperm storage.

Keywords: sperm storage, poultry, uterine fluid, Atomic Force Microscopy (AFM), metabolism

S4-0035 Genetically modified chicken model of RIG-I reveals antiviral innate immune mechanism

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RIG-I is a pattern recognition receptors of double-stranded RNA virus. It plays a significant role in the innate immune response. RIG-I is absent in chickens, the genetically modified chickens has posed a significant challenge due to the limitation of transgenic technology in poultry. The genetically modified chickens of the RIG-I were generated in this study can be reveal the role and mechanisms of RIG-I involved in innate immune. Using recombinant lentivirus-mediated gene transfer technology to generate RIG-I transgenic (TG) chicken, the integration and expression of RIG-I is analysis by PCR, Southern Blot, Western Blot; CEF cells of F1 TG are cultured and infected with the H5N1 avian influenza virus, PB2 gene, TCID50 and RIG-I downstream molecules were analysis by Real-time PCR, IFA to reveal RIG-I to inhibit influenza virus activity; In P3 laboratory, H5N1 infect in 4-week-old F1 TG and wild-type (WT) chicken, the role of anti virus is evaluate by the death time and virus titers; the gene expression and protein of each group chicken are analysis by iTRAQ and Illumina to clarify poultry antiviral innate immune mechanism. The results show that the TG chicken can stable genetic to F3 generation; The copy number of PB2 and TCID50 in TG are significantly lower than in WT group, the expression of IFN- β and Mx significantly increased. These indicated that RIG-I signaling pathway is constructed and innate immune response is induced in the CEF. Death time is delay and virus titers are significantly reduced show that RIG-I can inhibit the role of influenza virus; 495 proteins and 7466 genes are obtained by proteome and transcriptome analysis, 89 genes associated with proteins are identified, GO function analysis showed that proteins involved in innate immune response, inflammation, antigen presentation and cells adhesion. The project provides a basis for control of viral replication and spread and development of strategies of antiviral drugs and vaccines.

Keywords: RIG-I; transgenic chickens; innate immunity; proteome, transcriptome

S4- 0036 RNA-seq reveals differential gene expression in chicken testis with high and low sperm motility

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Sperm motility, one of the most widely used semen quality parameters, shows strong correlation with cock's fecundity. Previous study confirms many environmental factors can affect chicken sperm motility, including temperature, feed minerals deficiencies, and lighting regime. The heritability of chicken sperm motility was estimated higher than 0.5 by multiple studies, suggesting that genetic factors play an important role. The molecular mechanism underlying this trait is not well-understood yet. In the present study, RNA-seq was used to investigate the testis transcriptome of extreme sperm motility phenotypes in six Beijing- You chickens of 43 weeks of age: three birds with an average sperm motility of 8.4 and three birds with an average sperm motility of 1.8. The differentially expressed genes in the two group were scanned for further functional and pathway analyses. In total, 11,560 transcripts and 9,824 genes were identified averagely. Compared to high sperm motility group, up-regulation of 314 genes and down-regulation of 339 genes were observed in low sperm motility group ($P \leq 0.05$). Functional annotation analyses revealed that the up-regulated genes were mostly involved in metalloproteinase activity, negative regulation of protein metabolic process, extracellular matrix organization, and cell cycle process. The down-regulated genes belong to several specific biological functions related to guanyl-nucleotide exchange factor activity, regulation of ion transport, ATPase activity, spermatogenesis and other biological responses. Many significant differentially expressed genes have been proven to be associated with sperm motility in chicken or other species, such as EFCAB6, SPAG17, KIF14, KIF16B, and DYNC2H1. This study provides valuable information about the expression profiles of genes from chicken testis, and in-depth functional investigations of these genes could provide new insights into the molecular networks of sperm motility regulation in chicken testis.

Keywords: chicken, sperm motility, RNA-seq, transcriptome

S4- 0037 About the avian UF proteome: exosomes significance and biological processes in regard to eggshell mineralization, immunity and reproduction

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In avian species, spermatozoa are stored for long periods within the female reproductive tract. Oviducal environment influence during sperm storage remains unknown. An extensive description of the Gallus gallus uterine fluid (UF) proteome will help to provide the basis for a better understanding of long term sperm survival. UF was collected from 10 hens 10h after oviposition. Bottom up proteomic approach using SDS-PAGE and nano LC-MS/MS (ultimate 3000 RSLC system coupled to LTQ Velos Orbitrap mass spectrometer) was performed with a high-low resolution MS strategy. Data were matched against NCBI nr database using Mascot 2.3 and identifications were validated by the peptide and protein Prophet algorithm using Scaffold 4.0 software. Bioinformatic treatment of data set was carried out to refine annotation of proteins using NCBI nr database, and to describe UF proteins using SecretomeP 2.0 and SignalP 4.1 tools, InterProScan software, and, Exocarta, KEGG and UniprotKB databases. Among a total of 913 identified proteins, 160 were known to be secreted and 640 were matched against exosome databases. Thus we isolated UF exosomes by ultracentrifugation and analyzed by TEM (<100 nm) and western blot (exosomal markers, HSPA8, VCP). Molecular functions analysis of UF proteins revealed a majority of enzymes (34%) involved in glycolytic pathway and lipid homeostasis. Moreover, an exploration of biological processes indicated that 55 proteins had previously been described to be implicated in reproduction, 38 in mineralization and 85 in immunity. In conclusion, the UF represents a complex proteomic environment in which the presence of exosomal proteins may represent a novel and exiting mechanism of oviduct-spermatozoa interaction that may explain at least in part, the long term sperm survival. We expect and believe that the thorough catalogue of proteins presented here will be an interesting tool for biomarkers discovery involved in fertility.

Keywords: proteomics, bioinformatics, uterine fluid, poultry, sperm storage

S4- 0039 Improvement of medium composition by the addition of growth factors for the expansion of bone marrow-derived cells in chicken

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This study was conducted to enhance the efficiency of culture medium for expanding chicken bone marrow-derived cells (BMCs). Four-day-old white leghorn chicks were employed for BMC donor and the isolated BMCs were cultured in DMEM, to which 5 ng/ml bFGF and 500 unit/ml LIF were supplemented or not. To monitor the capacity of cell maintenance in vitro, primary cell attachment, CFU-F colony number, proliferative capacity, cell morphology, senescence assay, gene expression and differentiation potential were evaluated as experimental parameters. PLOC-GLM model in SAS package was employed for statistical analysis of these experiments. The number of CFU-F-positive colonies was higher ($p < 0.05$) after the bFGF and LIF addition than after no addition, which resulted improved primary cell attachment. In total cell population, better proliferative capacity was detected in the growth factor supplementation, whereas in senescence assay, a significant increase in the number of SA- β -Gal positive cells were also counted in the growth factor-containing than in growth factor-free condition. Osteogenic differentiation was apparently detected regardless of the growth factor supplementation, whereas adipogenesis was not significant after differentiation induction. Different expression profile in the expression of several pluripotency- or differentiation-related genes was detected after the supplementation or not, which differed from that of primordial germ cells and embryonic fibroblasts. In conclusion, the combined addition of bFGF and LIF to culture medium improved the culture efficiency of chicken BMCs of mixed population, which may contain various reprogrammable cells. This research was supported by Bio-industry Technology Development Program (IPET312060- 5), Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

Keywords: bone marrow-derived cells, cell culture, chicken

S4-0040 Influence of interval of artificial insemination on production performance in layer breeders

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The present study was conducted to research the effects of different artificial insemination (AI) interval on the production performance in Yukou Jing Pink I layer breeders. Sixty-four thousand breeders at 22 weeks were randomly divided into two groups (test group and control group), each with two replicates of 16,000 hens and 196 roosters. All the birds were raised in the same environment, provided the same feed and management. The test group adopted an AI interval of eight days, while the control taken an interval of six days. A series of data, including the rate of laying, fertility and mortality, were recorded from twenty-two to sixty-three weeks. No significant differences were observed for all the traits. Therefore, increasing the interval from six-days to eight-days did not differ in egg production (85.5% VS 86.0%), fertilization rate (95.6% VS 95.3%) and mortality (8.0% VS 7.8%). Adopting the eight-days AI interval directly reduced the number of roosters needed, increased the efficiency of AI, labor saving, and indirectly benefit the breeder producers in the long term production.

Keywords: AI, interval, egg production, fertilization rate, mortality

S4-0041 Effects of body weight on production performance in Yukou Jing brown breeders

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The present study was conducted to research the effects of different body weight on the production performance in Yukou Jing brown parent breeders. Eleven hundred and fifty-two Yukou Jing brown parent breeders of twenty week-old were chosen and divided into six groups with four replicates (48 birds). The groups were set by body weight as group I, II, III, IV, V, and VI (body weight range as 1460-1545g, 1546-1631g, 1632-1717g, 1718-1803g, 1804-1889g, and 1890-1975g). Data of the age of first laying, mortality, rate of laying, eggs per hen-day and hatching eggs per hen were collected from 23 to 53 weeks. The results were as follows: the age of first egg in group I was significantly later than the other five groups ($P<0.05$). The mortality in group VI was the worst ($P<0.05$), while no significant difference were found within group I to V. The rate of laying of group IV, V, and VI during the observing period were relatively higher than the other three groups ($P<0.05$), with no significant difference within. And the eggs per hen-day and hatching eggs per hen from group V and VI were obviously higher than the others. These results evidently indicated that body weight was associated with the production performance of Jing brown breeders, with an optimal body weight ranging from 1718-1889g at twenty weeks.

Keywords: body weight, production performance, Jing brown breeders

S4-0042 In vitro interaction of spermatozoa with hen's sperm storage tubules

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In birds, spermatozoa can be stored in specialized epithelial structures named Sperm Storage Tubules (SST) located at the utero-vaginal (UV) level. SST allow sperm cells to keep their fertilizing capacity several weeks. The aim of this work was to characterize in vitro the relationship between the SST cells and spermatozoa. So, we set up a primary SST cell culture method and co-incubated SST cells with spermatozoa. The utero-vaginal junction of hens (*Gallus gallus*) was dissected. UV fragments were enzymatically digested with 1µg/ml collagenase IX and 5µg/ml pronase in F-12 Ham overnight at 4°C. SST were isolated on 2%-4% Percoll gradient column. The culture was performed in 4 wells plates or in Lab-Tek Chamber Slide System with medium TCM 199 plus 10% BFS and 10 mg/ml Gentamicin. Cultures were maintained for 1, 5 and 8 days. Roosters sperm samples with more than 50% viability were pooled, seminal plasma was removed by centrifugation, then, spermatozoa were washed and resuspended with TCM 199. One million spermatozoa were added to each SST cells wells for 24 h at 37°C in 5% CO₂. We observed that in vitro SST have the epithelial morphological characteristics expected for immunocytochemistry: cytokeratin positivity and vimentin negativity. After 5 days of culture (i.e. at 5 and 8 days), we detected a lower signal for villin protein (1:500, Polyclonal Antibody, Bioss Inc.) on SST cells, most probably indicated less microvillousities on cell surface. A higher number of sperm cells bind to SST fragments in suspension (i.e. 1 day culture) than to SST cells monolayers (i.e. 5 and 8 days cultures). We speculate that the SST cells microvillousities may have a critical role in epithelium-spermatozoa contacts and their decreasing number for prolonged culture time may compromise sperm attachment. The model of culture we set up is a very powerful research system for a better understanding of SST-spermatozoa interactions.

Keywords: sperm storage tubules, SST culture, villin, spermatozoa

S4- 0043 Effects of different mating methods on embryonic development of indigenous chicken eggs

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Reproductive success in poultry depends to a very large extent on the efficiency of embryonic development during incubation of eggs. A study was conducted to determine the effects of different mating methods on embryonic development of indigenous chicken eggs. A total of ninety, 17 week- old normal feathered indigenous chicken breeders comprising 72 hens and 18 cocks were allotted to three mating methods: Pen mating (PM), Alternate males (AM) and Stud mating (SM). Each group, comprising 24 hens and 6 cocks, was replicated thrice with 8 hens and 2 cocks per replicate. One hundred and thirty-five hatchable eggs obtained from the hens were used for determination of embryonic development. Yolk, albumen and embryonic weights were determined on days 1, 7, 10, 15 and 18. Data obtained were subjected to analysis of variance in a completely randomized design. At day 7, eggs obtained from SM group had significantly ($p < 0.05$) higher albumen weight than PM with corresponding values of 34.13g and 27.19g whereas yolk weight was higher ($p < 0.05$) in PM (13.45g) than AM (11.31g). The residual yolk on day 15 of incubation was significantly ($p < 0.05$) heavier in SM (10.70g) than AM (8.96g) and PM (8.59). The values of 14.36% and 13.70% obtained for percentage moisture loss on day 18 of incubation in AM and PM respectively were significantly ($p < 0.05$) higher than 11.91% found in SM. Embryonic weight was not significantly ($p > 0.05$) influenced by mating methods throughout the incubation periods. It was concluded that the rate of albumen consumption by developing embryo became rapid between the 10th and 15th days of incubation.

Keywords: Mating methods, embryonic development, indigenous chickens

S4- 0044 Lentiviral vector mediated In- ovo silencing of ACC and PPAR in liver and adipose tissues of broilers

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The most important function of Acetyl Co-A Carboxylase (ACC) is to provide the malonyl CoA substrate for the biosynthesis and metabolism of fatty acids biotin carboxylase. Peroxisome proliferator-activated receptor- γ (PPAR γ) gene, is regarded as a “master regulator” of adipocyte differentiation and lipid storage influencing fat deposition in chicken. Two GFP lentivectors clones carrying shRNA for PPAR γ (PPAR γ - 1 and PPAR γ -2) and ACC (ACC-1 and ACC-2) were got synthesized from Sigma- Aldrich. The incubating broiler eggs (Ed 16) were in-ovo injected through broad end of the egg. A total of 18 eggs / group were injected with ACC- 1(4000 TU) + PPAR γ -1(4000 TU), ACC-2(4000 TU) + PPAR γ -2(4000 TU) and NTC (4000 TU) lentivector. The chicks hatched out of these eggs were reared up-to 6-weeks and then killed following the animal ethics guidelines and biosafety measures. Liver and abdominal adipose tissues were collected from 3-5 chicks/group. RNA was isolated from individual samples and subjected to analysis of PPAR γ and ACC gene expression analysis by real time PCR. The mean DD-Ct for PPAR γ gene in liver were 0.032 \pm 0.011, 0.041 \pm 0.011 and 0.113 \pm 0.038 for the respective shRNAs. The corresponding values of mean DD- Ct for ACC were 1.35 \pm 0.391, 1.020 \pm 0.575 and 4.040 \pm 0.729. The injection of ACC and PPAR γ specific shRNA exhibited significant down regulation of ACC and PPAR γ genes over the NTC. However, expression of PPAR γ in adipose tissues was not significantly downregulated. ACC gene did not show any expression in adipose tissue.

Keywords: shRNA lentiviral vector, ACC, PPAR γ , broiler, gene silencing

S4- 0045 Vitrification and transfer of poultry gonads to preserve heritage breeds in Canada

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Vitrification and transfer of gonad are important techniques for the preservation of the poultry resource in the world. Only a few group reported the success of regeneration of a poultry breed based on these techniques. The objective of this study is to adapt vitrification and gonadal transfer for the preservation of the poultry breeds produced in Canada. Two breeds were used for this study: Barred Plymouth Rock (donor) and a commercial line (recipient). Testes and ovaries from the donor line were vitrified by immersing the tissue into VS1 (7.5% DMSO and ethylene glycol) for 15 min, into VS2 (15% DMSO and ethylene glycol and 0.5M glucose) for 2 min, and plunging into liquid nitrogen. At the day of surgery, vitrified gonads were warmed into successive solutions of glucose (from 1M to 0M; 5 min of incubation in each solution). These tissues were transferred into a recipient within 2 hrs from the warming procedure. Recipient chick received MetaCam (0.015 mg) orally before the surgical procedure. Partial removal of the recipient gonad was performed before orthotopically introducing the vitrified- warmed gonad. After the surgical procedures, the recipient received mycophenolate mofetil daily for 3 weeks, and weekly for another 6 weeks (100 mg/Kg). At sexual maturity, potential grafts were retrieved and genotyped to confirm that the tissue was not related to the recipient. Our results demonstrated that we could not obtain a success of gonadal growth using ovarian graft (0 out of 5). However, success was obtained using testis graft (2 out of 5). Testis grafts had a full spermatogenesis as structurally matured spermatozoa were observed and these sperm cells were motile. These results confirmed that it is possible to regenerate a poultry breed using a genetically unrelated recipient line. However, it requires an optimization of the procedures to achieve success, which cumulate to an efficient preservation of poultry breeds.

Keywords: vitrification, transfer of gonad, preservation

S4- 0046 Effect of zeolite coated with silver nanoparticles on meat quality attributes of broiler chickens during cold storage

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Nanotechnology is quite competent and these materials may be used for assurance of food safety in different food products. This study was carried out to assess the effect of zeolite coated with silver nanoparticles on meat quality attributes of broiler chickens during cold storage. A total number of 375 one-day-old broilers (Ross 308) from a commercial hatchery were randomly assigned to 5 experimental groups in a completely randomized design (CRD). Experimental diets were following: 1) Basal diet (Control), 2) Basal diet supplemented with 1% zeolite 3, 4 and 5) Basal diet supplemented with 1% zeolite coated with 25, 50 and 75ppm nanosilver respectively. The birds fed diets containing various levels of nanosilver coated on zeolite (NZ25, NZ50 and NZ75) had lower MDA concentrations than the control group on d 3 after storage in the refrigerator ($P < 0.05$). Thigh meat water holding capacity (WHC) on d 7 after storage was significantly affected by the dietary treatments. In conclusion, findings of present study showed that dietary supplementation with zeolite coated with silver nanoparticles improved the oxidative stability of broiler meat during cold storage in the refrigerator.

Keywords: broiler, nanosilver, meat quality, zeolite

S4- 0047 Effects of artificial insemination on exosome localization and its protein level in sperm storage tubules of hen oviduct

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Avian sperm are stored in the sperm storage tubules (SSTs) in the hen oviduct for a prolonged period. However, the precise mechanism by which sperm can be kept alive in SSTs is still not fully understood. The aim of this study was to determine whether exosome is secreted by SST cells and play a role in the survival of sperm. The utero-vaginal junction (UVJ) of laying hens with or without artificial insemination (AI) were collected, followed by preparing frozen section and total protein extraction. The localization of exosome marker CD63 was determined by immunohistochemistry, and its protein level in the UVJ containing SSTs was examined by western-blot. The CD63 were localized in the apical part of UVJ mucosal epithelial cells and SST cells of both control and AI groups. The density of CD63 decreased in SST cells and tend to appear in SST lumen when sperm were stored in SST. The CD63 protein (approximately 40 kDa) was detected by western blot, and its level in UVJ was significantly higher in AI group. These results suggest that exosome were synthesized by SST cells and may be secreted into SST lumen when sperm were stored in SST. The exosome may be one of the factors that contribute to sperm storage function of SST.

Keywords: oviduct, sperm storage tubules, exosome, sperm survival

S4- 0048 Signaling pathways involved in chicken embryonic stem cell differentiation into male germ cells

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(Purpose) This study aim to study the expression of key signaling pathways and its' regulation mechanism in the process of chicken Embryonic stem cells differentiate to the male germ cell. (methods) The key signaling pathways in the process of ESCs towards the male germ cell differentiation was screened by RNA-seq technology; TGF- β and Notch signal pathway were selected to analyze its expression changes in chicken ESCs, primitive germ cells (PGCs) and Spermatogonial Stem cells (SSCs); At the same time, Western blot, qRT-PCR, Flow Cytometry and Immunofluorescence method were used to detect the reproductive marker gene in the process of germ cell differentiation in vivo and in vitro when the pathways were suppressed. (Results) Here, we used RNA-seq technology to first sequence chicken ESCs, primitive germ cells, and spermatogonial stem cells. Our results identified TGF- β and Notch signaling as key pathways involved in male germ cell differentiation. We next inhibited TGF- β and Notch signaling in chicken eggs. In eggs with inhibited TGF- β , expression of SMAD2 and SMAD5 was significantly decreased versus the control. Additionally, reproductive cells were significantly reduced in number. C-KIT, CVH, DAZL, STRA8, and integrin 6 were significantly down-regulated. When Notch signaling was inhibited, NOTCH1 was significantly down-regulated, but the percentage of germ cells increased significantly versus the control. In these cells, CVH, DAZL, STRA8 and integrin 6 increased significantly. We next used BMP4 to induce ESC differentiation into male germ cells. We found that SMAD2 and SMAD5 were significantly up-regulated versus the control and inhibited groups. The production of germ cells was also significantly increased versus the control group, but not the inhibited group. Expression of Notch1 was significantly down-regulated in these cells. (Conclusion) Our results indicate key roles for TGF- β and Notch signaling in chicken ESC differentiation into male germ cells.

Keywords: chicken embryonic stem cells, primitive germ cells, spermatogonial stem cells, RNA-seq, TGF- β signaling, notch signaling

S4- 0049 A new molecular test for male fertility diagnosis: Intact cell MALDI-TOF MS on sperm

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Evaluation of sperm quality based on the in-vitro measures of sperm cells' functions is often poorly correlated with fertility. There is a consequent need of alternative diagnostic tools both in veterinary and human medicine. We discovered recently that Intact Cell MALDI-TOF MS (ICM-MS) could be useful for in-vitro male fertility testing, since ICM-MS profiles from chicken fertile/subfertile spermatozoa were significantly different. In order to confirm this, a larger standardized experimental process was performed, and chicken sperm cells intact cell MALDI-TOF MS profiles acquired through a fast, automated method were employed to construct fertility-predictive mathematical models using data-dependent algorithms. This intact cell MALDI-TOF MS-based method showed high diagnostic accuracy in identifying fertile/subfertile males in a large male population of known fertility that included two highly different genetic lines (meat and egg lines). The identification of approximately 40% of the biomolecules represented in these spectra revealed that intact cell MALDI-TOF MS profiles typically comprise degradation products of proteins implicated in the most important functional pathways in sperm such as energy metabolism, structure and movement. Moreover, our results suggested that our ICM-MS method allows for the simultaneous analysis of molecular species implicated in different and important sperm functions related with fertility. We thus demonstrated that, with the use of appropriate models, intact cell MALDI-TOF MS can be used for accurate male fertility testing in the chicken, opening new opportunities for fertility diagnosis in any species.

Keywords: sperm, birds, fertility, phenotyping, diagnosis

S5-0002 The effects of cholecalciferol and 1 alpha-hydroxycholecalciferol supplementation on the development of tibial dyschondroplasia in broiler chickens

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This experiment was conducted to determine whether dietary cholecalciferol will alleviate a calcium and phosphorous (Ca-P) deficiency when 1  -OH-D3 is supplemented, and to determine the effects of adequate and inadequate Ca-P when 1  -OH-D3 is supplemented and vitamin D3 is adequate. A total of 144 one-d-old broiler chicks (Ross 308) were randomly allocated to three treatments. The dietary treatments were as follows: T1: adequate Ca-P + cholecalciferol and 1  -OHD3; T2: inadequate Ca-P + cholecalciferol and 1  -OHD3; T3: inadequate Ca-P + 1  -OHD3. The Ca and P levels in the adequate diets were 0.90% Ca, 0.616% tP; 0.75% Ca, 0.551% tP; 0.695% Ca, 0.508% tP for the starter, grower and finisher periods, respectively. The Ca and P levels in the inadequate diets were 0.80% Ca, 0.56% tP; 0.65% Ca, 0.50% tP; 0.595% Ca, 0.458% tP for the starter, grower and finisher periods, respectively. All diets were mixed with 500 FTU/kg of phytase and 5   g/kg of 1  -OH-D3 and cholecalciferol was provided in 5000 IU/kg except for regimen 3 that fed diet without vitamin D3. At 42 d, broilers were inspected for incidence and severity of tibial dyschondroplasia (TD). Contrast comparisons of data were carried out. The results showed that inadequate Ca-P supplementation with cholecalciferol significantly decreased the incidence of TD (60%), score (0.6) and tibia ash (39.97%) compared with broilers fed the same diet in the absence of cholecalciferol (TD: 75%; score: 0.6; tibia ash: 44.63%). The broilers fed inadequate Ca-P with cholecalciferol were unable to achieve the same tibia ash (39.97), and the incidence of TD (60%) as chicks fed Ca-P adequate diet (tibia ash: 43.42; incidence of TD: 40%). In conclusion, this trial suggests that broiler fed an inadequate Ca-P diet with 1  -OH-D3 and adequate level of cholecalciferol is unable to sufficient bone formation. There was no indication that 1  -OH-D3 in the absence of cholecalciferol was effective in reducing TD where as it could improve tibia ash.

Keywords: cholecalciferol; 1  -OH-D3; broiler; tibia ash; tibial dyschondroplasia

S5-0004 Effect of different energy to protein ratio diets on nest level choice in broiler breeders

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It has been shown in literature that providing diets with different energy to protein ratios to breeder pullets results in adjusted behaviour. Therefore, an experiment was conducted to investigate the effect of different dietary energy to protein ratios on nest level choice in broiler breeders. A 2 × 3 × 2 factorial design was used with 2 dietary protein levels (high = CPh; low = CPL) during rearing; high, standard and low dietary energy levels (3,000 = MEh1; 2,800 = MEs1; 2,600 = MEI1) during the first phase of lay; and standard and high dietary energy levels (2,800 = MEs2; 3,000 = MEh2) during the second phase of lay. A total of 2,880 one-day old Ross 308 BB female chicks were placed in 36 pens (4.5 × 2.5 m) with wood shavings and an elevated floor (1.2 × 2.0 m). Per pen, four laying nests (94 × 33 × 35 cm) on two levels (10 and 50 cm from slatted floor) were available to the hens. Breeders were fed via an automatic pan feeding system (5 pans) and water was supplied by one bell drinker above the slatted floor. The upper nest was accessible via a perch (6.5 × 4.5 cm) in front of the nest (15 cm from the nest). The number of eggs, prior to egg collecting, was counted at 27, 38, 46, and 54 wk of age. The REML variance component analysis procedure was used to analyse the effect of different diets. Observations of the different ages were analysed as repeated measures. The dietary treatments did not affect nest level choice. Contrary to expectations, among treatments breeders laid significant more eggs (81.3 vs. 18.7%) in the upper than in the lower nest. An interaction between nest level and age on nest level choice was found. At 27 wk of age more (85.8%) eggs were collected from the upper nest than at 38 (77.0%) and 54 wk (80.6%) of age whereas week 46 was intermediate (81.9%). It was concluded that different dietary energy to protein ratios did not affect nest level choice, whereas the upper nest was highly preferred during the entire laying period by the breeders.

Keywords: broiler breeders, diets, nest, level, behaviour

S5-0005 Using incubation temperature to manipulate broiler leg strength – an assessment in two commercial broiler lines

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The present study assessed the effect of incubating eggs at lower egg shell temperatures (EST) during the first 16 days of incubation, than the recommended EST of 37.8 °C, on the leg strength of two different lines of broilers. From previous research involving a fast feathering broiler dam line a lower incubation EST generated increased chick bone ash (BA) at hatch and extended standing times of the 35 day old bird, in a latency to lie (LTL) test. The current experiment assessed these same incubation ESTs on leg strength in two commercial broiler lines (Line A and Line B). Eggs from both lines of broilers were incubated under either standard EST (37.8 °C from sett until day 18 embryonation (D18E)) or slow start EST (starting at 36.9 °C at sett, gradually increasing to 37.8 °C at D16E). At D18E all eggs were transferred into individual cells in hatching trays and held in one incubator. From 468hrs (19.5 days) until 516hrs (21.5 days) incubation, eggs were observed every 6 hours to identify individual chick hatch time. All chicks were removed from the incubator (take-off (TO)) at 516hrs incubation. Chick weight and femoral bone ash (BA) were determined at TO. At 35 days of age the duration of standing in all male birds was assessed in a LTL test. Irrespective of incubation EST, Line A chicks hatched earlier than Line B. At TO, irrespective of line, early hatching chicks (from eggs incubated under standard EST), had significantly lower body weight than later hatching chicks (incubated under the slow start EST). Overall BA was significantly lower in Line A than Line B chicks at TO, but the slow start EST generated improved BA in Line A chicks. When 35 days of age, birds of both lines incubated under the slow start EST remained standing for significantly longer than birds incubated under the standard EST. In conclusion, broiler leg strength can be altered through incubation EST, but this effect may vary depending on the line of broiler.

Keywords: broiler, incubation, egg shell temperature, leg strength, locomotion

S5- 0006 Beak trimming: bar, template, welfare and technical results

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Beak trimming may cause deviations in beak form. These deviations may influence technical results and welfare. Traditional trimming may be done on a bar or through a template. In this experiment the effects of both are compared. 1080 Isabrown layers were kept on the floor (1/3 slatted, 2/3 litter; 45 individuals per pen) and trimmed at 6 weeks. In two pens the hens remained untrimmed. Hens were fed ad lib till 6 weeks after that hens were fed on guidance of weight. At 16 weeks beaks and plumage were measured. All results were compared by analysis of variance. Trimming was effective: no cannibalism occurred in the trimmed hens. In the untrimmed hens cannibalism was present in one pen. In the first week after trimming food intake and growth (as percentages of untrimmed hens) were lower in hens trimmed on a bar than through a template. This suggests that welfare is lower immediately after trimming on a bar. In the first 16 weeks no significant influence on growth, food intake and uniformity between hens trimmed on a bar or through a template was found. At 16 weeks the general appearance of the beak was significantly more deviant after trimming on a bar. In the lower beak "wild flesh" and little wounds had significantly higher scores after trimming on a bar. In the upper and lower beak was more weakness after trimming on a bar. This suggests that also later in life welfare was lower after trimming on a bar. However, plumage condition at 16 weeks was not significantly different. From the viewpoint of welfare trimming through a template seems better and no effect on zootechnical results was found. However, the larger project has shown that the more the beaks are destroyed the better the protection against severe feather pecking and cannibalism. A more elegant solution to diminish cannibalism could be efficient selection: see Van Rooijen, *Frontiers in Genetics* 5: 266 doi:10.3389/fgene.2014.00266 <http://journal.frontiersin.org/article/10.3389/fgene.2014.00266/full>

Keywords: beak trimming; bar; template; welfare; zootechnical results

S5- 0007 Awareness and practice of biosecurity measures applied in poultry farming in Kuwait

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The poultry industry in Kuwait is one of the most important animal production industries and its sales value exceeds \$ 100 million annually. The high susceptibility of poultry to disease outbreaks makes a comprehensive biosecurity technology a necessary practice in poultry farms to protect the farms and may cause irreversible economical losses to the poultry industry. Therefore this study evaluated the awareness and practice of biosecurity measures applied in poultry farming in Kuwait. The results indicated that the poultry companies are implementing a biosecurity program in all farms. However, the company's programs were modified and improved using the update regulations and measures related to biosecurity program worldwide. The result shows that the most practiced biosecurity measures in the study area are the ones relating to regular cleaning of the isolation, traffic control, and sanitation procedures. Also the result shows that the level of education, farm size, formal training in poultry production have significant positive influence on the farms' biosecurity control. The study therefore recommends among other things the intensive sensitization of the poultry farmers through workshop and field days seminars by appropriate agents on the benefit of adhering strictly to biosecurity measures on their farms. These recommendations were divided into two sections. Section 1: step-by-step follow tips, and Section 2: educational and warning biosecurity signs. It is important to follow all regulations recommended by the biosecurity program and implement them carefully in the farm to succeed in the protection from diseases. It is extremely important that poultry industry in Kuwait implement a comprehensive biosecurity program in their farms to ensure better quality production and to maintain the safety of poultry from biological hazards.

Keywords: biosecurity measures, poultry farm, Kuwait

S5-0008 Beak trimming: age, breed, blade speed, beak quality and technical results

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Working in a hurry is said to decrease beak quality. With a traditional debeaker one reason could be cracks caused by a high blade speed. In this experiment we have compared two standardised blade speeds. In practise blade speed is controlled by foot, in this experiment by air pressure. 900 brown and 900 white layers were trimmed (individually, not from a bunch of hens in one hand) with a Liefra debeaker at day 1, 8 (through a template) and at day 42 (on a bar), 360 hens of each breed remained untrimmed. Hens were housed in pens (1/3 slatted floor, 2/3 litter) with 45 animals each. Results were compared with analysis of variance. The highest delay in growth (as percentage of untrimmed hens) in the first week after trimming at day 1 and 8 was found at normal speed, at day 42 at high speed. However, on average no significant influence was present. No explanation was found. In both breeds during rearing no significant influence of blade speed was found on growth, food intake, uniformity and mortality. During the laying period no significant influence on egg production was present. Surprisingly hens trimmed at day 1 with normal speed have significantly less grown between 16 and 42 weeks than all other hens. Trimming at day 1 and 8 with high speed resulted at 16 weeks in significantly more symmetrical beaks, at day 42 in beaks with significantly more deviations. However, no significant influence on plumage condition at week 42 was found. Trimming on a young age or with a higher blade speed seemed no problem. Breed had no influence. A not investigated aspect of trimming in a hurry, trimming from a bunch, presumably has a negative effect on symmetry. The larger project has shown that the more the beaks are destroyed the better the protection against severe feather pecking and cannibalism. A more elegant solution to diminish cannibalism could be efficient selection: <http://journal.frontiersin.org/Journal/10.3389/fgene.2014.00266/full>

Keywords: debeaking, age, breed, blade speed, beak quality, welfare, technical results

S5-0009 Behavioural inventory and welfare status of young layers under different managemental conditions during winter season

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Behavioural responses and physical conditions of layers indicate the welfare and degree of adaptation to the production environments. The purpose of this study was to record the behavioural inventory of young layers of White Leghorn (CARI-Priya) and their welfare status under different managemental conditions during winter season (8- 150C). At 18thwk, a total of 450 layer birds from a single hatch were randomly assigned into three treatment groups viz floor housing, colony cage and individual cage. Continuous focal sampling method over a period of 24hrs by using CCTV cameras (marking the hens with different colours) was used to measure the time spent (% of 24hrs) in performing different behaviours (ingestive, comfort, walking, serotype, investigating etc). Feather condition, claw length, foot condition and keel bone deformity was studied in ten birds each selected randomly from housing systems by using suitable scale of measurement at 20th wk. Results revealed that, during winter, the floor reared layers spent significantly more time in sitting (46.4%), walking (7.2%) and investigating behaviour (4.0%) than those reared on colony (38.2, 4.8 and 1.4%) or individual cage (30.9, 1.4 and 1.0%). Whereas, individual cage layers spent more of time in feeding (40.7%) than the colony (26.6%) or floor birds (22.9%). There were significant differences ($P<0.05$) in the welfare status regarding feather conditions, claw length, gait scores and tonic immobility of the birds between different managemental conditions, while no significant difference was found in keel bone condition and foot lesions. Significantly higher ($P<0.05$) fearful response and time for first peck (response to novel object test) was observed in individual cage layers than the floor and colony birds. It is concluded, that the floor reared chicks are found to be more engaged in different behavioural activities than the cage birds. The welfare status of laying birds was compromised under cage conditions.

Keywords: behaviour, welfare, layer, housing, colony cages, winter

S5- 0010 A form of trimmed beak with, on a thin mash layer, a higher intake per bite than untrimmed

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The influence of the beak form of trimmed layers on food intake per bite on a thin or a thick layer of mash is investigated. Thousand hens were trimmed in various ways. The beaks were scored at 250 days. At 44 weeks all combinations (if present) were selected: short/less short (the mean distance between nostril and beak point was 8.3 mm in short and 13 mm in the less short beaks, in the untrimmed beaks 18.4 mm), equal/unequal (the mean distance between the point of the upper and lower beak was respectively 5 mm and 0 mm), weak/not weak (weak beaks are sensitive to touch), open/closed (one can look from the front in open beaks). For each combination of traits 4 hens were chosen. The combination weak and less short did not occur. 24 experimental and 24 company animals were individually housed in battery cages (in visually isolated pairs with a similar beak). After 24 hours of food deprivation (for maximum intake per bite) a trough with 250 g (5 cm) mash was placed in front of the hens. Number of bites was counted during 30 min. Food was weighted at beginning and end. This procedure was repeated. The whole procedure is also repeated with a 50 g (7.5 mm) mash. Analysis of variance was used. No signs of food spillage. All but one investigated trimmed beak forms increased feeding duration on mash and thus decreased the opportunity for feather pecking. A less short beak with a longer lower beak decreased feeding duration on mash compared with the other forms of trimmed beaks, thus increased the opportunity for feather pecking. On a thin layer feeding duration was even lower than in untrimmed beaks. Over 250 days after trimming effects on feeding behavior after deprivation were still present. The larger project has shown that the more the beaks are destroyed the better the protection against severe feather pecking and cannibalism. An elegant solution to diminish cannibalism could be efficient selection: <http://journal.frontiersin.org/Journal/10.3389/fgene.2014.00266/full>

Keywords: beak trimming, beak form, feeding behavior, intake per bite, mash layer

S5- 0011 Study of broody behavior pattern and comparison of biochemical indices between broody hens and laying hens

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The paper aimed to study the broody behavior pattern of Beijing You Chicken (BYC), a native bird, and compare some biochemical indices between broody hens and laying hens. 30 hens with sign of broody behavior and another 30 without sign of broody behavior were chosen and assigned in two floor litter pens. One trained staff was arranged to observe the birds twice a day at the feeding time (06:00~07:00, 13:00~14:00) from 43 to 50 wks, each time half an hour per pen. At 50 wks of age, 23 still broody hens and 23 still laying hens were sampled, and serum Prolactin (PRL), luteinizing hormone (LH), 17- β -oestradiol (E2), total protein (TP), lactic acid (LA), triglyceride (TG), total cholesterol (T-CHO), glucose (G), free fatty acid (FFA), total anti-oxidative capability (T-AOC), total superoxide dismutase (T-SOD) and malondialdehyde (MDA) were measured. The data were analyzed statistically using SPSS 15.0 Software for Windows. One-way analysis of variance and Turkey's test was used. $P < 0.05$ was regarded as statistically significant. The results showed that the number of characteristic broody hens was less than that of the non-characteristic broody hens (11/30 < 19/30), the number of two-time broodiness was bigger than that of one-time broodiness among the characteristic broody hens (7/11 > 4/11). The average duration for one-time broodiness is longer than that of two-time broodiness (22.7 > 18.6 d). PRL and LH concentration of broody hens were significantly higher than those of laying hens ($P < 0.05$), E2 had no significant changes ($P > 0.05$); TP and LA concentration in broody hens were significantly lower than those in laying hens ($P < 0.05$), and serum MDA concentration in broody hens was significantly higher than that in laying hens ($P < 0.05$). The study suggests that there existed significantly different behavior pattern and hormonal changes between the broody hens and laying hens, and there might be more oxidative stress in the broody hens than in the laying hens.

Keywords: broody behavior, biochemical indices, Beijing You chicken

S5-0012 Effects of toe claw removal on growth and blood biochemical indices of young male chickens during brooding-growing period

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The purpose of this study was to investigate effects of claw removal on roosters grown during brooding-growing period. A total of 600 1-day-old Hy-Line brown roosters were divided equally into 4 groups. Group 1 chicks served as the intact control. Toe clipping of group 2 chicks was performed by the method of claw reduction of the third toe; in group 3 chicks, the first and third toes; in group 4, the second and fourth toes. Body weight, tibia circumference and shank length of all chicks were measured at biweekly intervals from 2 to 12 weeks and 18 week. Four groups of 15 blood samples in each were collected to monitor blood biochemical indices at 42 days. The results showed gradual upward trends in body weight, tibia circumference and shank length with the increase of age. Meanwhile, claw removal had a significant effect on these parameters, which exhibited slight differences in group- and age-specific manners. Statistical results of blood biochemical indices displayed that, compared with the intact control, the content of glucose was significantly higher in groups 3 and 4, while the contents of total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol were significantly lower in group 3. Serum calcium concentration and relative activity of alanine aminotransferase were substantially increased in groups 3 and 4, respectively. No differences were found on the other measured indices like triiodothyronine, thyroxine, total protein, albumin, globulin, albumin/globulin, blood urea nitrogen, phosphorus, creatine kinase, alkaline phosphatase and aspartate aminotransferase between control and detoeing groups. In conclusion, our results suggest that toe claw removal had significant effects on growth and development of chicks in declawing number- differential and age- specific manners. These findings are expected to provide a theoretical basis for production improvement and welfare amelioration in breeders.

Keywords: toe claw removal, growth, blood biochemical indices, brooding-growing period

S5-0013 Long-term effects of rearing layer chicks with dark brooders on welfare, laying behavior and production performance

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Chicks require heat to maintain body temperature during the first weeks after hatch. This may be provided by dark brooders; i.e. horizontal heating elements equipped with curtains. The aim was to test long-term effects of rearing layer chicks with dark brooders on plumage and skin condition, mortality, laying behavior, and egg production in layers. Four brooder treatments differing in the size of the brooder (Large or Small) and in the management of the height of the brooder (Movable or Fixed) were tested. Groups of 102- 103 Isa Warren chicks were reared either with one of the 4 types of brooders (n = 4 per treatment, i.e. 16 in total) or with whole house heating (control: n = 6). At 16 weeks of age the group size was reduced to 50 individuals. Plumage and skin condition were scored at age 7, 16, and 28 weeks. Data on egg production and mortality was registered daily. We found that brooder birds had a better plumage condition throughout the experiment ($P < 0.001$) and fewer wounds on the body in the laying period ($P < 0.001$). Mortality due to other causes than cannibalism neither differed between treatments during the first 2 weeks of age (4.3%; $P = 0.73$) nor during the remaining rearing period (day 15-112: 0.6%; $P = 0.35$), whereas a difference was found during the laying period (day 113-199: 0.4% vs. 5.7%; $P < 0.001$). An outbreak of cannibalism occurred, but mortality due to cannibalism was significantly lower in the brooder treatments (day 113- 199: 0.6% vs. 7.9%; day 113- 199: 5.8% vs. 29.4%; $P < 0.001$). During the period from 17- 28 weeks of age brooder groups laid significantly fewer floor eggs (12.8% vs. 33.8%; $P < 0.001$) and had a higher total egg production (2393 ± 25 vs. 2108 ± 19 ; $P < 0.001$). We conclude that rearing with dark brooders can be a successful method of reducing injurious pecking and the prevalence of floor eggs, whereby mortality due to cannibalism is reduced, leading to improved welfare, egg quality, and production performance.

Keywords: dark brooder, cannibalism, egg production, laying hen, plumage condition

S5- 0014 The optimum sex ratio of the quality chicken in natural mating cage

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In recent years, escalating labor cost led to the increasing cost of the artificial insemination technology for breeding stock raising. The natural mating technology has been regarded again mainly due to the fact that the low-cost and the advocating of animal welfare. The aim of this study was to investigate the optimum sex ratio of the quality chicken in natural mating cage. In this study, a total of 96 cocks and 912 hens were randomly selected and divided to four big groups, the sex ratio of group one is 1:7, group two is 1:8, group three is 1:9, and group four is 1:10, and then divided each big group to six parallel groups, marked as 1, 2, 3, 4, 5 and 6. The test duration was 16 weeks. The results showed that the fertility rate of group one was 93.1%, that of group two, group three and group four was 91.4%, 88.9% and 84.3% individually. The economic benefit analysis show that the comprehensive income of group one was 5424 yuan, that of group two, group three and group four was 5727 yuan, 5588 yuan and 5287 yuan individually. Comprehensive results show that the fertilization rate of the group two can reach more than 90%, and the overall egg yield is the highest. Therefore, the optimum sex ratio of natural mating patterns of the quality chicken is 1:8.

Keywords: quality chicken, sex ratio, natural mating cage

S5- 0015 Gene expression profiling of HTR2C in Dongxiang blue-shelled layers

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Feather pecking (FP) is a serious behavioral disorder of laying hens. FP is a multi-factorial problem caused by both genetic and environmental factors. Feather condition score was used to assess plumage condition because of the relation to feather pecking activity. The HTR2C is one of a family of receptors that is involved in coordinating the intracellular responses to serotonin in the mammalian nervous system. A SNP of the chicken HTR2C gene showed significant association with higher FP performing in layers. Dongxiang Blue-shelled layer is a Chinese local layers with blue-shelled eggs, and are vivacious aggressive. In this paper, gene expression profiling of HTR2C was detected in different tissue including heart, liver, spleen, kidney, skin, pectorals, crureus, hypothalamus, opisthencephalon and cerebrum of Dongxiang blue-shelled layers(N=8) using RT-PCR. The level of HTR2C expression in different part of the opisthencephalon and cerebrum(left and right) in the layers with significant different feather condition scores(N=12) was also compared. The results showed the HTR2C expressed in all the tissue collected, and the highest expression of HTR2C presented in kidney. After comparing the HTR2C expression in different part of the opisthencephalon and cerebrum(left and right) in the layers with significantly different feather condition scores, the expression is significantly higher in the right cerebrum of layers with poorer feather condition scores. The results are suggesting that the chicken HTR2C were also involved in multiple pathways, and the right cerebrum is the main part which affects feather pecking.

Keywords: gene expression profiling, HTR2C, Dongxiang blue-shelled layers, feather pecking

S5-0016 Effects of stock density on the welfare of laying hens in floor pens

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The effects of stock density on the laying performance, egg quality, blood biochemistry, corticosterone levels, and bone mineral density of laying hens were investigated. Eight-hundred 34-week-old Hy-Line Brown laying hens (*Gallus gallus domesticus*) were randomly assigned to one of 4 treatments, each of which was replicated 4 times. Four stocking densities, including 5, 6, 7, and 10 birds/m², were compared. A commercial-type basal diet was formulated to meet or exceed nutrient recommendations for laying hens from the National Research Council. The diet was fed to the hens ad libitum for 8 weeks. Results indicated that increasing stock density decreased hen-day egg production. Egg mass and feed intake also decreased, while oviposition rate and broken egg production rate increased with increasing stock density. During the initial 4-week (34 to 37 week) and final 4-week (38 to 41 week) periods, serum corticosterone increased with increasing stock density as well. Lastly, while bone mineral content was not affected, bone mineral density decreased with increasing stock densities. These results indicate that increasing the density beyond 5 birds/m² elicits some negative effects on laying performance of Hy-Line brown laying hens.

Keywords: bone mineral density, corticosterone, laying performance, stock density, Hy-line Brown laying hens, animal welfare

S5-0017 The effect of balanced reduced protein diet on the feed pecking behavior of broiler breeder hens

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The impact of balanced reduced protein concentrations in broiler breeder diets are currently being investigated with potentially positive outcomes such as normal reproductive performance, but improved persistency of egg production, better fertility and hatchability. However, data on the effects of such diets on breeder behavior are scarce. Therefore, an experiment was initiated to see if the long term feed restriction and feed composition have effects on the feed pecking behavior of broiler breeder hens. Feed pecking frequency and rhythm were followed by focal sampling during the feeding time in certain weeks for the two groups (control diet and 25% reduced protein diet). Lower protein group receive 10% more feed amount than control group to reach up to the same standard body weight. In week 23, 26 and 29, the control hens pecked more than the low protein at the beginning of the feed provision but pecked less at the end of feeding time. These observations suggest that control birds have more appetite at the beginning of the meal but are more satiated at the end of the meal. The 10% extra amount of feed for low protein group might provide an explanation for their higher pecking frequency or rhythm at the end of feeding time as well. However, in week 33 and 38, no significant differences were found anymore between the two groups of hens at the onset of the meal. In contrast, hens fed the reduced protein diet displayed a significant lower pecking behavior at the end of the meal. These hens were also characterized by a lower feed intake rate towards the end of the daily meal. With increasing egg production, the feed amount for breeder hens is upgraded accordingly. By combining the results of two groups, pecking frequency and pecking rhythm increased during the daily feeding time in week 23 and 26 but decreased in week 29, 33, 38. Appropriate explanation needs to be further investigated.

Keywords: broiler breeder hens, reduced protein diet, feed pecking frequency, feed pecking rhythm

S5-0018 A comparison of nesting behaviour in RFID- based single and family nests

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The aim of the study was to evaluate the impact of two nest systems on the nesting behaviour of layers. Therefore, Lohmann brown layers were housed in two barn compartments with different RFID-based nest systems, Weihenstephan funnel nest boxes (FNB, n=72) and high frequency group nest boxes (HFGN, n=16). Both nest systems use RFID-transponders to register the nesting behaviour. Whereas, only one layer at the very same time can use the single nest (FNB), the family nest (HFGN) is occupied with up to ten layers. Therefore, only in family nests social behaviour between layers can be observed. More than 850 layers were reared together and transferred to the production barns at an age of 18 weeks. Taking into account the age of the hen, the layers were divided into two groups, each group in one system. Data recording started at an age of 21 weeks. After 4.5 months the groups were changed and housed for further 5.5 months in the other system. The number of occupations and the duration of a nest visit were compared for both systems with Wilcoxon signed rank sum test. In summary, the effect of familiarisation was seen in each system. During the first month of observation in a specific nest system, the number of nest visits per hen was higher compared to the 2nd to 4th month. In 28 days, a layer had on average 3 more nest visits in the FNB, when housed there first, though the number of nest visits was 29 for both nest systems when housed in the HFGN first. During the whole observation period the variation in the number of nest visits was slightly higher for the HFGN than for the FNB. The duration of a nest visit was on average 6 minutes longer in the HFGN than in the FNB, irrespective of the nest system in which the layers were housed first. Therefore, the layers show a very homogenous nesting behaviour in single nests, where they seem to feel free of external factors. In family nests the same layers show a more variable behaviour which results in better nest acceptance.

Keywords: nesting behaviour, RFID, single nest box, family nest

S5- 0019 Effect of feeding system, genotype and gender on behaviour and stress in broiler chickens

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The present study aimed at evaluating whether and how behaviour and stress of broiler chickens could be affected by genotype (standard vs. high breast yield), sex, and feeding regime (ad libitum vs. restricted rate, 80% from 13 to 21 d of age). A total of 768 day-old chicks was housed in 32 pens, their behaviour was video-recorded at 11, 18, 25, 32, 39 and 45 d of age, and controlled for 10 consecutive seconds every 30 min to measure the percentage of chickens showing mutually exclusive behaviours (standing, sitting/lying, walk, drink, feed, peck floor, peck fixture, peck tail of other bird, peck other bird, peck own tail, dust bath). Faeces pooled by pen and individual plasma were weekly sampled to measure corticosterone concentrations by microtitre radioimmunoassay (RIA). Behavioural and corticosterone data were analysed with PROC GLIMMIX and MIXED (SAS Institute, Cary, USA), respectively, including genotype, gender, feeding regime and age as fixed effects, and pen as a random effect. On the whole trial, genotype affected the percentage of standing chickens (11.1% vs. 12.3% in standard vs. high breast yield; $P<0.001$). Males were observed pecking other birds more than females (0.11% vs. 0.05%; $P<0.001$) and showed less comfort activities (6.21% vs. 6.51%; $P=0.05$). Broilers submitted to feed restriction were more active than those fed ad libitum (standing birds: 12.5% vs. 10.8% ; $P<0.001$), were more often at the feeders (9.19% vs. 8.20%; $P=0.01$), and showed higher faecal corticosterone (13.6 ng/g vs. 12.2 ng/g; $P<0.10$). In conclusion, feed restriction promoted broiler activity, but chickens experienced hunger and stress (as measured by corticosterone concentrations), whereas the other factors had a weak effect. Relatively to stress measurements, corticosterone in plasma was not affected, whereas pooled faeces collected from the litter appeared to be a valid matrix, to be sampled in a repeated, non-invasive, and non-stressful way.

Keywords: feed restriction; welfare; broiler chickens

S5-0020 Impact of post-natal transport duration and parental age on broiler chick welfare and performance

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The impact of post-natal transport on chick welfare and production is still poorly understood. The effect of transport likely depends on the chicks' quality, which is influenced by parental age. The aim of the study was therefore to assess the effect of post-natal transport duration and parental age on chick welfare and performance. After hatch, 3240 mixed-sex chicks from either 29-wk-old (young) or 60-wk-old (old) breeders were transported for 1.5h (short) or 11h (long). After transport, 2800 chicks were divided over 100 pens. On average 6 chicks per crate (n=228) were selected to assess chick quality, body weight (BW) and yolk sac weight (YW) at d1. After culling, blood was collected and assayed for stress parameters. Average daily gain (ADG) and BW were recorded until d41. No interaction effect of parental age and transport duration was found for any of the variables. Chick quality was lower in chicks from old versus young breeders ($P<0.001$). BW ($P<0.001$) and YW ($P<0.0001$) at d1 were lower for chicks from young breeders compared to those from old breeders, and for chicks transported for 11h compared to the 1.5h-transported chicks (BW: $P<0.01$; YW: $P<0.0001$). The BW-difference between parental age treatments persisted until slaughter, and ADG was higher for chicks from old breeders ($P=0.017$). Transport did not affect performance (BW until d41, ADG). Long transport resulted in lower plasma lipid peroxidation ($P<0.001$), but higher corticosterone levels (CORT, $P<0.001$) compared to short transport. Lactate ($P=0.063$) and glucose ($P=0.077$) levels tended to be lower after long transport. Chicks from young breeders had higher CORT levels than those from old breeders ($P<0.001$). Parental age affected chick welfare, quality and performance. Long transport caused a stress response, as indicated by reduced glucose and lactate, and elevated CORT levels. However, there are no indications that 11h-transport in Belgium impose long-term performance risks.

Keywords: day-old chicks, transport, welfare, breeder flock age, broilers

S5-0021 How much dual purpose survived in traditional breeds compared to modern hybrid lines?

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Dual purposed breeds gained social and political importance to avoid the killing of male chicks in layer lines. Whether traditional breeds or modern lines can be used is influenced by their performances, but these data are rare. In addition, it is not known how performances of traditional breeds developed under a half century of show breeding. We compared traditional breeds (Bergischer Schlotterkamm, German Reichshuhn) and the hybrid Lohmann Dual according to their body weight gain, laying performance and carcass composition. We also were interested in behavioral traits that favor the adaptation to the environment. Therefore, we run experiments to investigate exploration (open field), fear (tonic immobility), memory (novel object recognition) and learning (discrimination). A total of 59 individuals of both sexes were tested in a quasi-experimental design with a special focus on age- and repetition-related effects. Data were collected via an online tracking program (Viewer, Biobserve) and analyzed analytically (SPSS, Systat). We found differences in growing rates in terms of a high sexual dimorphism in hybrids. Start of lay was up to 11 weeks later in traditional breeds with comparable egg weights. Carcass composition differed with larger thighs and higher exploitation values in hybrids. Explorative behavior was pronounced in hybrids. Tonic immobility response was slightly increased when chickens turned sexually mature but did not differ in principle. Working memory processes found did not show major differences in the level of neophobia. Learning behavior reveals an advantage for traditional breeds, which need fewer repetitions to reach criterion. In conclusion, we found Lohmann Dual to show better production traits than the traditional breeds investigated. In terms of relevant behavioral traits, they range between the two traditional breeds tested exhibiting an adaptive behavior towards their environment which favors animal welfare from the chickens point of view.

Keywords: hybrid lines, production performance, behavior, dual purpose, traditional breeds

S5- 0022 Effects of different beak treatment systems on layers performance

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Beak trimming is a routine husbandry procedure practiced in the commercial poultry industry, particularly in broiler and laying hens. Infrared Beak Treatment (IRBT, Nova- Tech Engineering Inc., Willmar,MN), which is an automatic beak trimming process for day-old chicks at hatchery, is more and more popular in recent years. However, little additional research is available on the effectiveness of IR trimming as an alternative method for use in production. Hy- line brown commercial layers(N=3000), which share the largest market in China, were selected in this study. The layers were treated by Nova-Tech IRBT machine with different module and intensity (25-23 and 27-23) at one day, comparing with no beak trimming and hot blade group (beak trimming using hot blade at day10). The layers were raised in an intensive multi-layer and environment control farm. Development and production performance were collected and analyzed regularly. The results showed that IRBT had a little influence to the early growth of the layer. The mortality at 10 day is 0.3% in IRBT with 25-23, which is higher than IRBT with 27-23(0.2%) and no beak trimming (0). The 2 wks body weight of the no beak trimming group was highest, and the IRBT with 27-23 was higher than the hot blade group. With developing, layers of IRBT with 27- 23 showed better growth performance. The mortality of laying period in group IRBT with 27-23 is almost 1% lower than the other groups .The feather score in week 72 of the group IRBT with 25-23 was 5% better than the group with no beak trimming. The egg quality was not affected by different Beak trimming methods. The results from the study suggest that infrared treatment with 27/23 can be optimized for superior productivity in the intensive multi-layer and environment control farm of China.

Keywords: infrared beak treatment, hot blade, beak trimming, layers performance

S5- 0024 Effects of separated- sex reared method on growth performance, feeding and drinking behavior of Ross 308 broilers

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The aim of the present study was to investigate the growth performance, feeding and drinking behavior of broilers with separated-sex rearing. A total of 960 one- day- old broilers (Ross 308) were divided into 3 treatments with 40 replicates (pens) per treatment, respectively. Three treatments were designed in this experiment: male group, female group and mixed- sex rearing group (one half was male chicks and another half was female chicks). The experiment lasted for 35 days and all birds were free to feed and water. Performance and behavior were measured systematically each week. Male broilers had significantly higher ADFI and ADG, and lower F/G compared with female broilers on all feeding phases ($P<0.05$). The separated-sex chicks presented better growth performance than mixed-sex group on day 35. Male birds spent more eating and drinking time during 0830 to 0930 and 1500 to 1600 hr than female birds ($P<0.05$). The feeding and drinking frequency of male birds was higher than female birds. Additionally, birds of both male and female groups during 1500 to 1600 hr spent more time in feeding (800 sec per hour) and drinking (116 sec per hour) than 0830 to 0930 hr (540 and 100 sec per hour) ($P<0.05$). These results suggest that separated-sex rearing had greater benefits than mixed- sex rearing. Therefore, single-sex flocks should be taken into account in future considerations of optimal maximum performance.

Keywords: separated-sex rearing, growth performance, feeding behavior, drinking frequency

S5-0025 The effects of light intensity in weeks 1 to 7 of rearing for chicks, and stressors consisting of combined transport, relocation and mixing in week 16, on plumage damage, social avoidance and mortality, in adult free-range laying hens

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Eight hundred day-old ISA Brown chicks (16 pens of 50 birds) were reared in a 2×2 factorial experiment with replication, to investigate the effects of light intensity (Low: 5 lux vs Normal: 40 lux) from 1-7 weeks, and the application of stressors (combined transport, relocation and mixing: TRM) at 16 weeks versus no TRM (Not TRM) on plumage damage, social behaviour and mortality between 16-41 weeks. Continuous access to outdoor runs occurred from 20 weeks. Plumage damage due to feather pecking was apparent from 18 weeks, and by 41 weeks more than 60% of hens were affected. Flock mortality was ~10%, mainly due to cannibalism. While there were no effects ($P>0.05$) of Lux or TRM on plumage damage, there were Lux × TRM interactions ($P<0.001$) on mortality, which was highest in the Low lux + Not TRM treatment and lowest in the Normal lux + Not TRM treatment. Lux and TRM influenced social avoidance, when tested at 38 weeks with 216 pairs of unfamiliar hens from the same rearing treatments. Although Low compared to Normal lux hens were quicker to leave the starting position ($P<0.001$) in the test arena, Normal lux + Not TRM birds were slowest ($P<0.001$) to come within 0.3 m of each other. Low lux hens were more likely to peck aggressively at the unfamiliar hen than Normal lux hens ($P<0.001$). One interpretation is that rearing chicks under low lux possibly influenced ocular development resulting in poorer visual acuity in the adults, contributing to reduced avoidance and increased aggression. Further, mixing unfamiliar birds as part of the TRM treatment may have also modified avoidance behaviours as adults, such that hens which experienced mixing at 16 weeks remained more cautious when approaching unfamiliar birds. Both factors potentially influenced social avoidance responses, which may be relevant to birds becoming targets for, or alternatively avoiding feather pecking, and associated plumage damage and cannibalism. Further research is clearly warranted.

Keywords: laying hen, light intensity, stress, free range, welfare

S5-0026 Effects of stocking densities on growth performance, economic benefit, and welfare parameters of broilers

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To study the effects of stocking density on growth performance, economic benefits, and welfare parameters of broilers from 21 to 35 d of age, total 540 of 21-d old male and female broilers were separated and reared in different steel-wire cages at size of 0.49 m² per cage, at the begin of this experiment there was no significant difference of body weight among different treatments. Densities were designed at 5, 7, 9, 11, and 13 birds per cage (25, 35, 45, 55, 65 kg/m² slaughter weight), respectively. Each density was included of 12 replicates (6 male and 6 female cages). Results showed that: 1) Compared to the densities no more than 45 kg/m², feed intake was significantly decreased at the densities of more than 45 kg/m² in male and 55 kg/m² in female broilers. There was no significant difference of ADG and FCR of both in male and female broilers among the densities of 25 to 45 kg/m², however the ADG was significantly decreased at the densities of 45 to 65 kg/m² than other low densities, and this decline of ADG was bigger in male broilers than female broilers. FCR was significantly higher at the densities more than 45 kg/m² in male and 55 kg/m² in female broilers. No significant difference of mortality of both female and male broilers was observed between treatments. 2) Economic benefit was increased when the densities increased from 25 to 45 kg/m² in male broilers and 25 to 55 kg/m² in female broilers; However Profit (yuan/m²) was reduced when the densities more than 45 in male and 55 kg/m² in female. 3) Footpad score was not significantly affected between different densities, however there was more leg problem at the density more than 45 kg/m² in male broilers and more than 55 kg/m² in female broilers.

Keywords: stocking densities, performance, economic benefit, welfare parameters

S5-0027 Danish achievements on foot pad lesions in broilers

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Very low prevalence of foot pad lesions (FPL) in broilers in Denmark. For years, the industry has worked determinedly and efficiently to reduce the prevalence of FPL. FPL is an important indicator of potential welfare issues and problems such as poor litter quality, poor animal health or imbalances in the feed that could affect the profitability of the production. FPL may range from mild redness to serious lesions. It is a result of many factors, the most important being exposure to poor litter quality with high levels of ammonia. Systematic FPL monitoring of all flocks at slaughterhouses became a legal requirement in 2001. From each flock, 50 feet from the first and the last part of the flock are examined. FPL is scored 0, 0.5 or 2 points according to the severity of FPL. The industry's intensive efforts have produced positive results. The general prevalence of FPL has decreased, including severe cases of FPL. 269 flocks were slaughtered in July 2015; 95% of the flocks had no FPL, 0% of the flocks had severe FPL. The remaining flocks had mild FPL. In 2003, 30% of the flocks had no FPL, 15% had severe FPL and 55% had mild FPL. The reduced prevalence of FPL has been achieved through increased focus on skilled management, including climate, feed, management of litter and drip-reducing nipples and by introducing technological solutions such as heat exchangers in broiler houses. In addition, the industry has offered complimentary advisory services to producers who had serious problems with FPL. Also, a private penalty system exists: payment will be reduced if a high prevalence of severe FPL is observed at slaughter. Conclusion: The outcome is improved animal welfare and higher profits, also because the feet have an export value. FPL has been successfully reduced by efficient management and technology improvements. FPL is an animal-based indicator of welfare, and the routine scoring of FPL has been instrumental in improving broiler welfare and health.

Keywords: animal welfare, broilers, foot pad lesions, management

S5-0028 Risk factors associated with dead on arrivals in broiler chicken transports

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The pre-slaughter phase is a critical phase of the production process with potentially serious animal welfare implications, including death. These deaths are usually termed as Dead on Arrivals (DOAs). In order to reduce DOAs, its risk factors ought to be known. Therefore, the study's aim was to examine the link between DOAs and potential pre-slaughter risk factors. Commercial transports (n=79) to 5 slaughter plants in Belgium were assessed. The transports consisted of $48,453 \pm 27,168$ birds aged 41.2 ± 1.3 d and weighing 2.58 ± 0.4 kg (all means \pm SD). Farmers provided information on the flock, and observers gathered data on pre-slaughter factors. Data on DOAs were recorded by slaughter plant personnel. First, associations between 51 potential risk factors and DOAs were tested using univariate models. Significant risk factors ($P < 10\%$) and non-significant, but relevant, factors were considered for inclusion in a multivariable linear regression model. The final model was selected based on the results of an automatic stepwise selection procedure. Median DOA% was 0.19% (range 0.04-3.34%). Multivariate analysis resulted in a model with 4 factors that explained 29% (R^2) of the variation in DOAs. Fewer DOAs were found when farmers checked day-old chick quality (0.21% vs. 0.51% when not checked; $P=0.012$), when birds were of low slaughter weight ($P=0.046$), when birds were manually caught by professionals (0.27% vs. 0.46% when caught by acquaintances; $P=0.01$), and when birds were laired inside (0.27% vs. 0.38% when laired outside; $P=0.045$). From our results, we conclude that improvements for broiler welfare could be made by adjusting management of the farmer. Additionally, birds should be lighter rather than heavier and caught by a team of professionals. Birds should be laired inside at the slaughter plant. Still, the precise, causal relationship between risk factors and DOAs should be further investigated to determine how DOAs can be reduced most efficiently.

Keywords: dead on arrivals, transport, welfare, broilers

S5- 0029 The effect of alternative feeding strategies during rearing in broiler breeders

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Because broiler breeders have the same genetic potential for fast growth as broilers, they are quantitatively feed restricted to reduce obesity related problems in fertility. This chronic feed restriction leads to welfare concerns over the birds' lack of satiety. The objective of this research was to examine the effect of rationed alternative diets and non-daily feeding schedules for broiler breeders under simulated commercial conditions during rearing. At 3 weeks of age, 1,680 Ross 308 pullets were conveniently allocated into 24 pens fed with one of four isocaloric treatments: (1) daily commercial diet (control); (2) daily alternative diet; (3) skip-a-day commercial diet; and (4) graduated commercial diet with varying on-feed days per week. Alternative diet had an inclusion of 40% soybean hulls and 1-5% calcium propionate, increasing with time. Subsamples of birds were weighed at 6, 10, 14, and 17 wk, scored for maturity at 23 wk, and scored biweekly for foot lesion and hock burn prevalence. Litter moisture was determined weekly. Data were analyzed with a mixed model procedure, with week as a repeated measure and pen as a random variable. Birds fed on skip-a-day and graduated schedules were lighter ($P<0.0001$) than control birds. There was also a lower percentage of mature birds in the skip-a-day vs. control treatments ($P<0.05$). While there was no overall effect of treatment on body weight uniformity ($P=0.34$), treatments differed in body weight uniformity over time ($P=0.007$). Litter moisture was higher ($P<0.001$) in pens fed the alternative diet compared to control, although differences disappeared after week 14. The prevalence of foot lesions was higher in birds receiving the alternative diet ($P<0.01$) compared to control; however, these differences were only significant in wks 5 and 7. Results from this research suggest that non-daily feeding schedules used with commercial stocking densities reduced feed efficiency without a significant increase in flock uniformity.

Keywords: broiler breeders, alternative feeding strategies, welfare, nutrition, rearing

S5- 0030 Effect of dietary methionine and lysine level on behavior of broiler chickens

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This study was conducted to investigate the effect of different level of dietary methionine and lysine on the behavior of broiler chickens. A total of three hundred and twelve 1-day-old Cobb X Cobb broilers were placed in the floor pen with six treatments with 4 replicates (pen) of 13 birds each for 35d. Three levels of dietary total methionine (Prestarter: 0.70, 0.63, 0.57%, Starter: 0.63, 0.57, 0.51%, grower: 0.57, 0.51, 0.45% and two levels of dietary total lysine (prestarter: 1.40, 1.25%, starter: 1.25, 1.10%, grower: 1.10, 1.00%) were added in the diet and supplied to the birds. Video was recorded weekly for 10 minute in each day and observe the behavior of resting (sitting, standing), moving (walking), consummatory (drinking, feeding), explorative (pecking) and instinctive (preening, flapping) behavior of broilers. Data were analyzed using the non parametric GLM statement of SAS. In pre-starting period, results showed that walking behavior was increased with the level of dietary methionine (0.70 %) and lysine (1.40 %) in the diet ($P<0.05$). Feeding behavior also increased with dietary methionine level in the diet. Therefore, drinking behavior was affected by the dietary methionine from 2 to 5 weeks of age ($P<0.05$). As the results, higher dietary methionine activates the walking behavior of broiler chicks. Thus, dietary methionine and lysine content would be able to influence the behavior of broiler chicken and need to apply for an optimum animal welfare direction.

Keywords: methionine, lysine, behavior, welfare, broiler chickens

S5- 0032 Signs indicating imminent death in *Escherichia coli* infected broilers

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Broilers were observed for signs indicating imminent death after inoculation with a virulent strain of *E. coli*. Broilers were inoculated intratracheally at 8 days of age with 107 *E. coli* 506 in 0,3 ml PBS per bird and observed until 17 days of age, when the experiment was terminated. Of these birds, 114 were observed from a distance of 1 - 2 meters each hour, and nightly by camera observation with respect to these 5 items: level of attention, locomotive activity, posture and appearance, interaction and impairment of respiration. For deviations of the normal state for these 5 items, i.e. clinical signs of disease, scores were defined ranging from 0 - 1 to 0 - 3. The periods of time elapsing from attaining a score for the first time to death were registered per bird for each score for each item. Of 114 birds, 86 did not present any notable sign of disease, 28 presented a clinical history: 25 died after presenting signs of disease, 2 died without previous signs, 1 was diseased but survived. When a bird presented signs of disease, it was subjected to extended clinical examination: temperature, heart rate, respiratory rate and capillary refill time were measured. These measurements proved of lesser predictive value. From signs visible at a distance of 1 - 2 meters, the attention and the posture and appearance were affected most often in diseased birds, 26/28 and 25/28 respectively; 25% of these died within 5 and 4 hours respectively, 50% died within 12 hours and 75% died within 20 and 19 hours respectively. It appeared that any sign of disease visible from 1 - 2 meters distance indicated imminent death, with 75% of the birds dying within 20 hours. From these observations a protocol for intervention to prevent animal suffering may be designed.

Keywords: broilers, clinical signs, dying, *E.coli*, predicting death

S5-0034 Improvement of robustness and performance in broiler chickens by short-term temperature training in the hatcher

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An alternative approach to improve functional traits alongside with production efficiency of fast growing broiler chickens provides temperature training in the hatcher (perinatal temperature training from day 18 until hatching, PTT) with short-term warm loads (+ 1° C, maximum 2 hrs/day). PTT of the developing thermoregulatory system during critical periods has long lasting effects on thermal adaptability and various body functions, because of the strong relationship between the central control of body temperature and functions, such as metabolism, feed intake and body weight regulation, immune and stress response. The hypothesis is that PTT improves robustness via reduction of the basic metabolism by long-lasting epigenetic metabolic programming. Hence, robust chickens have more energy available for performance as well as for adaptation, immune and stress responses during environmental challenge, which meets important animal health and welfare aspects. Broiler chickens were incubated under experimental (100-1000 eggs) and commercial conditions (115.200 eggs) using standard single stage incubation program (control) and PTT. In the PTT group the secondary sex ratio was changed in favor to male chickens, hatching rate and body weight of hatched chickens were increased, and chick quality (Pascarscore) was improved. During incubation, PTT reduced oxygen consumption with long-lasting metabolic effect (significant reduction) on hypothalamic neuropeptide Y expression. Random sampling of 120 males and 120 females from control and PTT group was used for subsequent growth trials of 35 days. PTT induced better feed conversion mostly along with better body weight gain. On day 35 the heterophile to lymphocyte ratio was significant lower in the PTT group. During acute stress, PTT chickens can mobilize more energy, characterized by higher T3/T4 level. In conclusion, in broiler chickens PTT may improve robustness alongside with production efficiency as a practicable epigenetic tool.

Keywords: broiler, incubation, robustness, performance, metabolic energy

S5- 0035 Identifying feather eating Isa Brown hens using artificial feather presentation

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Feather pecking and cannibalism are major welfare concerns for free range layer production. To determine the nutritional motivations behind feather pecking behaviour, feather eating birds must first be identified. A total of 202 individually caged 20-week old Isa Brown females were used in this study. Hens were initially presented with 5, but increased to 10 semi-plume and downy feathers measuring 4-6cm in length. Feathers were mounted on an artificial plastic substrate suspended in front of the cages on days 1, 3, 5, 8, 10, 15, and 17 of the trial. Feather removal was recorded 30m, 1h, and 2h after feather presentation. Individual behavioural observations were also performed on 59 randomly selected hens, with the latency to peck, number of feather pecking bouts, and the number of feathers pecked, pulled, and eaten in a 30 second period from the first feather pecking bout recorded. Latency to peck was limited to 30 seconds of feather presentation with observations stopping if birds did not peck within that time. On average, 57% birds removed all feathers within 2 h. Significant differences ($P<0.001$) in feather removal were found between birds, highlighting the individual nature of feather pecking behaviour. Only 66% of individually observed birds were seen to eat at least one feather during the observation periods, with the number of feathers eaten ranging from 1-10 feathers in a 30 second period. Latency to peck was significantly lower ($P<0.001$) for feather eating (FE) birds, with 50% of birds pecking at the substrate within 1 s, and 75% of birds pecking within 2 s. In contrast, non-feather eating birds (NFE) were only 25% likely to peck at the substrate within the first 4 s of presentation. Feather eating may not be the final outcome for all feather pecker bouts however FE birds have a much stronger motivation to peck than NFE birds. This trial identifies a potential feather eating bird population for study in further trials.

Keywords: cannibalism, feather eating, feather pecking, Isa Brown

S5-0036 Housing system affects neural plasticity and behavior in the chicken

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There is ongoing debate on the effect of housing systems on the well-being of laying hens including use of cages, modified cages, and aviaries. This study was conducted to determine effects of housing on neural development. Hy-Line W-36 chicks were housed from 1d to 34wk in a battery cage system (C, brooder, grower, layer cages) or in floor pens (F, enriched). At 36wk, half of birds in each system were left there and the other half were moved to the opposite system to create 4 groups (Floor-Floor, FF; Floor-Cage, FC; Cage-Floor, CF; Cage-Cage, CC). Five brains were sampled at each of 10 time points from 1-34wk and at 38, 42, and 45wk for measuring dendritic length (DL) of hippocampal (Hp) pyramidal cells; 5 brains were collected at 3 time points to assay brain-derived neurotrophic factor (BDNF). No differences in BDNF were found (5wk ($P>0.05$), though BDNF in F birds was numerically greater than C birds at each wk. DL increased from 1-34wk but no differences were found between the two housing systems in DL except at wk 5 ($F>C$; $P=0.0225$), one wk after C chicks were moved from brooder to pullet cages. At 38, 42, and 45wk, there were no differences between FF and CC brains ($P>0.05$) in DL. At 38, 42 and 45wk, DL in FC and CF brains was greater than FF and CC ($P<0.05$) but FF and CC were not different from each other ($P>0.05$); at 42wk, $FC>CF$ ($P<0.05$). Results suggest that as long as birds remain in the same environment in which they were reared, Hp DL development, a sensitive marker of neuroplasticity associated with environmental enrichment, is similar in both housing systems. Changes in DL appear to be associated with dynamic rather than static enrichments, regardless of enrichment. Behavioral tests (tonic immobility, TI; emergence time, ET) showed generally greater fear in C birds from 2-33wk; H:L ratios did not differ between F and C birds but C values were numerically greater at all but 2 time points, suggesting greater long-term stress in birds housed in cages.

Keywords: hippocampus, dendritic arborization, BDNF, housing environment, behavior

S5-0037 Analysis of the epizootic situation regarding productive poultry's ectoparasites in Ukraine

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At this time poultry is one of the most progressive livestock industries in Ukraine. Productive poultry has common infectious and parasitic diseases. Important role, among the parasitic diseases, has diseases that cause by parasitic insects. Their persistence among the livestock leads to reducing of poultry's productivity and it can outbreaks infectious and parasitic diseases. Ectoparasites are vectors and reservoirs of these pathogens. The goal of our work during the 2010-2015 was conducting of parasitological research at poultry farms. It was established persistence of temporary and permanent ectoparasites among the poultry. It was studied the epizootic situation at poultry farms. Production facilities, litter, equipments of the poultry houses were examined. It was identified by a microscope the ectoparasites' species. It was found that existence of the poultry's ectoparasites is a very big problem at small farms especially where, multi-age poultries are contained together and where is used floor keeping. The Mallophagoses which we identified from chickens were *Menopon gallinae*, *Menacanthus stramineus*, *Menacanthus cornutus*, *Goniocotes hologaster*, from turkeys – *Menopon gallinae*, *Menacanthus stramineus*, from ducks and geese – *Anaticola crassicornis*, from ostriches – *Struthiolipeurus struthionis*. It was established persistence of darkling beetles *Alphitobius diaperinus* from broilers and laying hens with floor keeping. Sporadically it was detected colony of red chicken's mite *Dermanyssus gallinae*. At the poultry farms where industrial technology is using the big problem is the red mite *Dermanyssus gallinae*. Mite's populations were detected in farms with ovigerous birds' crosses, quails', waterfowls' breeding. It was established strong tendency to increase of *Dermanyssus gallinae* at the industrial poultry sector of Ukraine. The problem with dermanissioz in Ukrainians' poultry is still going and requiring immediate decision.

Keywords: red chicken's mite, Mallophagoses darkling beetles, ectoparasites.

S5- 0038 Current situation analysis on Chinese chicken's drinking water

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To get a better understanding of the current situation of chicken's drinking water quality and to provide scientific evidence for monitoring its drinking water in China. The survey was conducted in 6 cities, and the survey population involved some main chicken-yield areas in china (Beijing, Guangzhou of Guangdong, Suining of Sichuan, Zhangzhou of Fujian, Nanchang of Jiangxi, Yulin of Guangxi) . The water supply and sterilization system in these six farms were investigated, and water samples collected from these farms were taken back to the laboratory for bacteriological and heavy metals detection. Tests were conducted under the directions of "Standard Test Method for Drinking Water" GB/T 5750- 2006, and the criterion of results was according to "Drinking Water Health Standards" GB/T 5749- 2006. The results showed that 1) All the farms did not have regular sterilization, bacterial and metals tests recently on chicken's drinking water, except the one of Nanchang's. 2) There was only one chicken farm in Suining city did not met the microbiological standard. The contents of thermotolerant coliforms and *Escherichia coli* were both higher than the recommendation of GB/T5749-2006 (< 2 MPN/100 mL). 3) Heavy metal contents in waters of Guangzhou and Nanchang cities were qualified for the standards .The concentrations of As in waters from Beijing, Suining and Yuning cities were significantly higher than the recommendation of GB5749- 2006 (< 0.001 mg/L). The contents of Hg of waters from both Suining and Yuning cities were significantly higher than the maximum allowance levels (< 0.002 mg/L). In summary, the drinking water in these areas has excessive amounts of bacteriological and heavy metals. More disinfection and filtration measures should be took to improve the quality of chicken's water in these places.

Keywords: chicken's drinking water, bacteria, heavy metals

S5- 0039 Effects of low- crude protein diets on growth performance, immune indexes, meat quality and welfare quality of free- range yellow broilers

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In China, there are many breeds of yellow meat broilers, which are divided into three types (fast growing, middle growing, slow growing) depend on their growth rate. So it is important to study the nutrient requirement according to different type and different raising system. Meanwhile with the increasing shortage of protein feed resource and people's concerning about the livestock excrement pollution, it would be significant for the sustainable development of broiler industry to reduce feed crude protein levels and the amount of nitrogen in livestock excreta. This experiment was conducted to investigate the effects of low crude protein(CP) diet on growth performance, immune indexes, meat quality and welfare quality of free- range yellow broilers from 22 to 56 days of age. A total of 540 21-day-old male Xueshan yellow broilers were randomly assigned to 3 groups with 3 replicates per group and 60 birds each replicate. The birds were fed with the diet containing CP 19%(control), 18%, 17% for 35 days. The results showed as follows: reducing dietary CP level had no significant effect on growth performance, immune indexes and meat quality($P>0.05$). Compared with the control group, foot pad dermatitis and nitrogen content in feces were significantly lower in the groups that chicks fed diets with lower CP($P<0.05$), and they were linearly affected by CP($P=0.014$, $P=0.038$, respectively). Meanwhile, the albumin and uric acid in serum showed significant response to CP($P<0.05$), and there were significant quadratic correlation between them($P=0.017$, $P<0.012$, respectively). In conclusion, reducing some low protein level of diets can improve welfare quality and environment condition without influencing growth performance, immune indexes and meat quality of free-range yellow broilers.

Keywords: free-range yellow broilers, low-crude protein, growth performance, welfare quality

S5- 0040 Neurobehavioral changes and tonic immobility response under heat stress condition in japanese quails supplemented with withania somnifera roots ethanolic extract

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This study was carried out to investigate the effect of Withania somnifera roots ethanolic extract (WSRE)70% supplemented to heat stressed (35~40°C) Japanese Quail on neurobehavioral changes (NC) and tonic immobility response (TI). The birds, divided randomly into 4 groups of equal numbers and located in isolated room. The first group was considered as control and administered only with normal saline while, the second and the third groups were administrated orally with root ethanolic extract which were dissolved in distilled water at a dose rate 50 or 100 mg/kg body weight the fourth group was received 0.5 mg/kg body weight diazepam. The behavior was recorded after 0.5,1,2,4 and 24 hours post administration for each group. Neurobehavioral changes according to open field arena test (OFAT) were: Onset of movement from middle square (sec), number of striping crossed within five minutes, number of jumping trials within five minutes, pecking order within five minutes and TI. Effect of oral administration of WSRE on NC in OFAT showed significant dose dependent effect in increasing time of onset of movement from middle square and in reducing number of jumping trials out box in comparison to untreated group whereas all supplemented groups(WSRE and Diazepam) show significant reduction in the number of crossed striping during all measurement times(except after 24 hrs); while oral administration with WSRE did not lead to significant reduction in pecking order in comparison with control group also result show significant increase in the time of TI compared with control. Oral administration with 0.5 mg/kg body weight of diazepam showed stronger effect in this test. We can concluded from NC study in OFAT and TI that WSRE could offer sedative effect on treated quails under heat stress condition.

Keywords: withania somnifera roots ethanolic extract, neurobehavioral changes and tonic immobility, open field arena test, heat stressed Japanese Quail

S5- 0041 Activity and occupation analyses as broiler behaviour and welfare indicators

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The activity and occupation patterns of broiler chickens are measured to check the performance and status of the flock using Precision Livestock Farming (PLF) technologies. The aim of this study is to illustrate how monitoring flock activity and occupation, provides retrievable information on their habits and activity patterns, which can be used to check the flock health and welfare status. Four top view cameras are used in a commercial broiler house in The Netherlands to visualize the floor area, continuously recording images during the light cycle periods which are translated into activity and occupation indexes per minute. The monitoring of these indexes through successive light periods reveals how behavioral patterns of the flock evolve during the cycle. By using the previous three light periods, it is possible to estimate the activity and occupation levels for the following period and evaluate deviations (20% or higher). These deviations are expressed as the percentage of time birds spent in an alert situation. This percentage shows a statistical relevant correlation ($p < 0.05$) with the welfare quality assessment scores. Thus, performing this analysis in specific areas defined according to the topology of the house shows how different activity or occupation patterns affects broiler welfare and behavior and helps the farmer in the daily management.

Keywords: broilers, behaviour, welfare, precision livestock farming

S5- 0042 Enthalpy index comfort and productive losses during broiler chickens transport

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Temperature and humidity are stressor agents to broilers chickens during transport to slaughter. Calculating thermal comfort in the loads allows understand animal welfare. The proposal was to evaluate chicken load microclimate underwent different seasons and distances and their effects on slaughter weight and mortality. 24 transport crates were monitored in 12 different loads where data loggers recorded temperature and humidity to calculate the Enthalpy Comfort Index (ECI), also considering barometric pressure. Crates with 7 broilers were weighted before and after transport to calculate body weight loss - BWL (kg). Death on arrival - DOA (%) was measured in the slaughterhouse. Fully randomized experimental design with 12 treatments, arranged in factorial with 2 (seasons: winter and summer) x 2 (distance: near and far) with three replications. The worst EIC were measured to the long distance (70.6kJ.kg-1) in the summer and to the near distance (50.5kJ.kg-1) in the winter. In the summer, BWL was higher for long distance (0.065kg) than near (0.033kg). In the winter, major BWL was observed to long distance (0.073kg) in comparison with close distance (0.045kg). Mortality was no different between far (0.19%) and close (0.15%) distance during the summer. In the winter (dried period), it was 0.22% for long distance and statistically superior ($P < 0.05$) to near distance (0.11%). ECI in the summer was critical to broiler transport; the interaction between summer and long distance resulted in lethal zone score in regard to thermal stress. The longest distances resulted in higher BWL for both seasons. BWL and mortality were higher in the winter. However, ECI has been ranked in the thermal comfort zone. In conclusion, there was no correlation between ECI and BWL and mortality rate. Worst EIC did not contribute to reduction broiler performance. The existence of thermal core in the load can be compromising broiler welfare than reflecting significant productive losses.

Keywords: body weight loss, microclimate, mortality

S5-0043 Effects of residual feed intake on shank and tibia characteristics in F2 chickens

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Increasing body weight associated with more feed intake and a variety of metabolic abnormalities such as skeletal disorders in chickens. Recently, residual feed intake (RFI) has been defined as a selection criterion to improve feed efficiency. The aim of this study was to investigate the effect of RFI on shank and tibia characteristics in chickens. A total of 126 male and female birds from an F2 population, originated from crossing between a slow-growing native fowl and a fast-growing commercial line in two hatches, were used. Shank and tibia lengths (mm), weights (g), diameters (mm), tibia breaking strength (kg/mm²) and ash content (%) were measured after slaughter of the chickens. Also, RFI was calculated based on regression of feed intake (FI) on average daily gain (ADG) and mid metabolic body weight (MMBW). All the birds were divided into two groups based on their RFI values. Mean and standard deviation of RFI for 25 percent of efficient (RFI-) and inefficient (RFI+) birds were -10.53 ± 4.2 and 9.5 ± 2.8 , respectively. While FI was significantly different in both RFI- and RFI+ groups ($P < 0.01$), ADG and MMBW were not different. Shank and tibia characteristics were significantly different between male and female birds ($P < 0.01$). However, the measured traits were not significantly affected by different hatches ($P > 0.05$). Shank and tibia lengths, weights and diameters were not significantly different between RFI- and RFI+ chickens ($P > 0.05$). Although breaking strength and ash content were lower in efficient birds than inefficient birds, these differences were not statistically significant ($P > 0.05$). Non-significant results for shank and tibia characteristics between two different groups of RFI values confirmed that RFI is independent of birds' growth traits and skeletal disorders and could be a good criterion for selection.

Keywords: residual feed intake, shank and tibia characteristics, F2 chickens

S5-0044 Systematic analysis of feeding behavior and their effects on feed conversion efficiency in Pekin ducks

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Feeding behavior has been considered as an important trait in animal production, however, no systematic studies had been conducted to investigate the relationship between feeding behavior and production. The relationship between feed conversion efficiency and feeding behavior is nearly unknown, due to difficulty of collecting a long term feeding data. This study aims to investigate the feeding behavior and their effects on feed conversion efficiency in Peking ducks. In this study, 2926, 7 groups of Pekin ducks were fed from 0 to 6 week. During 3-6 weeks, live weight, feeding time, feeding frequency, and feed intake were recorded using our own developed automatic equipment. Residue feed intake (RFI), feed conversion ratio (FCR), feeding time per day, feed intake per day, feeding time per count, feed intake per time of individual were calculated. Basic statistics about feeding behavior during recording period were analyzed. Rank correlation and Pearson correlation analysis between feeding behavior and feed conversion efficiency were performed. The results showed total feeding time and total feeding frequency have a significantly positive relation with RFI (correlation coefficient: 0.25~0.54 and 0.25~0.50, p-value<0.05). Feeding time per days and feeding frequency per day also have a positive relation with RFI (correlation coefficient: 0.03~ 0.30 and 0.109~0.314). Moreover, feed intake per time have a low-level relation with RFI. Nevertheless, the traits of feeding behavior have no significant relation with FCR ($P > 0.05$). The results illustrated the feeding behavior could be a potential aspect for improving feed conversion in duck breeding.

Keywords: feeding behavior, residue feed intake, Pekin duck

S5- 0045 Regulation of protein and gene expression in the hens' oviduct by corticosterone

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Stressful environment can affect protein abundance in egg white, in addition to reducing their performance in quality and quantity. The objective of this study was to investigate the effects of dietary corticosterone as a stress model on the expression of proteins and mRNAs in the oviduct of laying hens. After environmental adaptation, 2 groups of laying hens were provided for 14 days with either control (CON)- or corticosterone (CORT)- containing diet at 30 mg/kg. Magnum tissues of the oviduct dissected out on day 14 were determined for protein and mRNA expression using 2DE with MALDI-TOF/TOF-MS/MS and qPCR, respectively. Dietary CORT differentially affected expression of 45 proteins and genes ($P < 0.05$) with different biological roles including immunity and cytoskeleton. The current results, together with the results of our previous study in egg white, suggest that stressful environment can play an important role in the regulation of protein and gene expression in the oviduct of laying hens leading to modulating abundance of proteins in egg white.

Keywords: stress, laying hens, magnum, proteomics, differential expression

S6-0001 Effects of heat stress on somatostatin and some related immune factors in small intestine of Wen-chang chicks

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To investigate the effects of heat stress (HS) on developmental changes in immune functions of chicken intestinal mucosa, one-day-old broiler chicks were randomly assigned into control check (CK) and HS groups and raised under indoor temperature. The chicks in HS group were subjected to heat stress at $40 \pm 0.5^\circ\text{C}$ from 12:00 to 14:00 every day. Intestinal mucosa samples were collected at the end of weeks 1 to 6, and the effects of HS on somatostatin and its related immune factors were examined using immunohistochemistry, physiological and biochemical methods. The results showed that HS obviously increased the amount and integral optical density of somatostatin positive cells, somatostatin content as well as IFN- γ and IL-2 levels in small intestine, and these increases reached statistical significance in some intestinal segments ($P < 0.05$). In addition, IgG, IgA and IgM levels fluctuated in different intestinal segments and their levels in jejunum, duodenum and ileum in 6-week-old chicks were significantly lower in HS group than in CK group ($P < 0.05$). The contents of immune-related enzymes also fluctuated, but the activities of acid phosphatase, lysozyme and glutathione reductase in duodenum and jejunum were lower in 6-week-old chicks in HS group than in CK group, some reaching statistical significance ($P < 0.05$). Growth hormone (GH) and HSP70 contents in multiple intestinal segments in 6-week-old chicks were significantly higher in HS group than in CK group ($P < 0.05$). The results indicated that 1) HS could increase the expression and secretion of somatostatin and affect the normal development of immunoglobulins, cytokines and immune-related enzymes in chicken small intestine, thereby impacting the immune function of chicken intestine; 2) GH and HSP70 in small intestine are involved in self-protection mechanisms against HS-induced intestinal injury and somatostatin regulation may be one of the important components.

Keywords: heat stress, somatostatin, intestinal mucosa, immune factors, chick

S6-0002 Immune response of laying hens under chronic ammonia stress

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The aim of this study was to investigate the effect of ammonia (NH_3) on the immune response of laying hens. 192 hens of 25 week were divided in to the two treatment: hens with fresh air (control) and hens exposed to 30 ppm atmospheric NH_3 for 25 weeks. Plasma immunoglobulins (IgA, IgG, and IgM), complement factors (C3 and C4), acute phase proteins (AGP), albumin, interleukins (IL-1 β , IFN- γ and TNF- α), and splenic cytokine gene expression were determined (n=16). Chronic ammonia exposure to the hens led to a general state of stress as evidenced by increased H/L ratios ($P<0.05$) in treated hens compared to controls. There were no differences in albumin concentrations and Acute Phase Protein (AGP) between ammonia exposed group and control group ($P>0.05$). Chronic ammonia exposure to the birds could affect the immune function as evidenced by decreased IgM and C4 concentration levels ($P<0.05$). There were no differences in IgG, IgA, C3, IL-1 β , IFN- γ , TNF- α of plasma and splenic cytokine IL-1 β , IL-6 and TNF- α mRNA expression in chronic ammonia group versus control group ($P>0.05$). These findings demonstrated that ammonia exposure at 30 ppm suppress immunity of laying hens, as indicated by the changes of H:L ratios and plasma IgM and C4 concentrations.

Keywords: ammonia, stress, hens

S6-0003 Performance and carcass characteristics of heat-stressed broilers supplemented with choline

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The effects of supplemental choline (CHO) on broiler performance and carcass characteristics were studied under different temperature conditions: thermo-neutral (TN) and heat stress (HS). Four hundred day-old mixed sex commercial broilers were weighed, wing tagged and placed in each environment. Eight replicates of 10 chicks were randomly assigned to one of five dietary treatments: Treatment 1 (CON) = basal diet; 2 (CHO500) = as 1 plus 500 ppm methyl equivalents added CHO; 3 (CHO1000) = as 1 plus 1000 ppm methyl equivalents added CHO; 4 (BET500) = as 1 plus 500 ppm methyl equivalents added BET and 5 (BET1000) = as 1 plus 1000 ppm methyl equivalents added BET. The corn-soybean basal diet was formulated to meet NRC requirements for broilers. Feed intake and body weights were recorded weekly. Litter samples were collected from each pen to evaluate wetness and feet assessed for footpad health on day 42. At slaughter on day 52, breast meat was collected and drip loss evaluated 4 and 7 days post slaughter. Breast meat lightness (L^*), redness (a^*) and yellowness (b^*) were measured. Data were analyzed using mixed model ANOVA (SAS 9.3, Cary, NC). Feed intake was reduced ($P<0.05$) for HS birds during days 21-35 and 36-49 (20 % and 29 % respectively). Similarly, HS birds had lower ($P<0.05$) weight gain during days 21-35 and 36-49 (24 % and 31 % respectively). There was a diet X temperature interaction ($P=0.04$) on drip loss 4 days post slaughter. The lowest drip loss occurred with CHO500 (0.60%) in HS birds. Breast meat color of HS birds was significantly ($P=0.02$) lighter (54.21) while that of TN birds was significantly ($P=0.004$) more yellow (3.75). Temperature did not affect footpad dermatitis ($P=0.22$) however, there was an effect of diet ($P=0.003$) with CHO500 and BET1000 showing the lowest occurrence. In this study, breast meat drip loss was influenced by dietary CHO and BET while color was affected by rearing temperature.

Keywords: broilers, heat-stress, choline, footpad dermatitis

S6-0004 Effect of laying hen house with long length to ventilation mode in winter

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With the expansion of the scale of breeding, large-scale laying hen house is becoming longer and longer, but recommended length is no more than 80m. This research explored the ventilation mode selection of house with long length. It was conducted in a 6 stacked empty laying hen house with 130m in length, and compared the airflow distribution in cross ventilation mode, tunnel ventilation mode with minimum ventilation volume (165,600m³/h), mix ventilation mode with maximum ventilation volume in winter (399,600m³/h). The results showed that in the house with 130m in length in minimum ventilation volume, in tunnel ventilation mode, wind speed in front of house was too small (0.03m/s), while it was too high at the end of house (0.92m/s), average wind speed were (0.48 ± 0.28) m/s and (0.34 ± 0.26) m/s at height of first and 5th cage, respectively. In the same amount of ventilation volume, when ventilation mode changed to cross ventilation, the uniformity of airflow distribution increased dramatically, wind speed in most part of house was less than 0.3m/s, average wind speed were (0.15 ± 0.14) m/s and (0.08 ± 0.04) m/s at height of first and 5th cage, respectively. In mixed ventilation mode with maximum ventilation volume, average wind speed were (0.38 ± 0.32) m/s and (0.26 ± 0.21) m/s at height of first and 5th cage, respectively. So for the house that is too long, in terms of airflow distribution, only with tunnel ventilation is not suitable, but in view of so much dead corner existing in cross ventilation, mixed ventilation mode is suggested. And the arrangement place of fan should be further researched.

Keywords: laying hen house, long length, ventilation pattern, winter

S6-0005 Evaluation of Vitamin E as heat stress alleviating agent in broiler chicken during hot-humid summer

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High environmental temperature exposure is of major concern for the poultry industry especially in hot regions of the world because of the resulting poor performance, immune-suppression and high mortality. It has been suggested that modern poultry genotypes produce more body heat, due to their greater metabolic activity. High humidity during hot humid condition aggravates stress further by mobilization and excretion of minerals and vitamins from tissues. So to address this issue and to evaluate efficacy of vitamin E as ameliorating heat stress agent a study was designed with 120 broiler chicks, reared in cages on a standard diet up to 14th day of age and thereafter up to 42nd day (14-42d) on test diets with or without vitamin E. Dietary treatment groups were three in number viz., T1 (control diet), T2 (vitamin E @ 150mg/kg) and T3 (vitamin E @ 250mg/kg). Each treatment comprise of five replicate of eight birds each. Experiment was carried out during hot-humid (August-September, 26.0±0.12 to 34.25±0.37°C, Rh, %: 76.95±0.90-86.15±0.61) summer. It was found that production parameter as well as energy and protein efficiency were improved significantly (P<0.001) in all supplemented group during all phases. Cellular as well as humoral immunity and lymphoid organ weight were improved (P<0.001) due to vitamin E supplementation during hot humid summer. The hemoglobin, total protein, AST and ALT were (P<0.001) improved, While H:L ratio, total cholesterol and serum corticosterone decreased (P<0.001) in vitamin E supplemented groups. Based on these result it was concluded that supplementation of vitamin E (@150mg/kg) will improve production, immunity and as well as stress biomarkers (H:L ratio, serum corticosterone) leading to improvement of welfare of birds during heat stressed conditions.

Keywords: Vitamin E, hot humid summer, immunity, physio-biochemical profile

S6-0006 Study on layer performances and egg quality characteristics among different tiers in the mating cage during the mid-late laying period

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The aims of the present study were to investigate the variations in light intensity, layer performances and egg quality characteristics among different tiers in the mating cage during the mid-late laying period, and further insight into the potential molecular mechanisms of tier level underlying this effect. All birds (Hy-Line Brown parent stock breeders) at 53 weeks of age were placed into 3-tier mating cage. The light intensity, layer performances and egg quality characteristics were measured among different tiers as well as the mRNA expression patterns of follicle-stimulating hormone receptor (Fshr) and luteinizing hormone receptor (Lhr) were evaluated in the hypothalamic-pituitary-gonadal axis (HPGA) in all birds using real-time polymerase chain reaction (PCR). Remarkable differences in light intensity were observed among different tiers, which imply potential disparity in layer performances and egg quality characteristics. Further analysis revealed that tier level had a significant effect on shell color, albumen height, Haugh units and yolk color, but no effect on egg weights, egg shape index, shell strength, shell thickness and percentage of yolk. The results of reproductive performances analysis displayed that tier level did affect the percentage of defective eggs and percentage of setting eggs, but did not affect laying rate, egg fertility, hatchability of fertile eggs, hatchability of setting eggs and mortality rate. In addition, real-time PCR analysis showed that Fshr and Lhr were expressed in the different HPGA regions. However, the relative expression of both receptors did not change substantially in the corresponding HPGA regions in all birds among different tiers. In conclusion, our overall results suggest that tier level may be a contributing factor for some layer performances and egg quality parameters.

Keywords: layer performance, egg quality, tier level, mating cage

S6-0007 Salmonella Enteritidis within different scales of layer farms in the Northern part of China: on-farm sampling survey results using PCR method

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Salmonella enterica subspecies *enterica* serotype Enteritidis (SE) has led to several outbreaks of food-borne disease for decades. It is transmitted mainly from contaminated eggs to humans. SE exists originally in laying houses, and interacts with environmental factors intimately. In this study, we aim at coming up with a viable PCR method to identify SE from environmental samples within different scales of layer farms, and evaluate the contamination status of SE of 4 main egg producing provinces in a voluntary SE survey study carried out in the Northern China between the month of August and December in 2014. In the first step, after specificity retrieval using Primer-BLAST against NCBI database, 3 out of 8 SE specific oligonucleotide primers from previously published papers were selected as candidate primers. The primers targeting *Prot6e* gene were adopted and primers targeting *Sdf I* were also selected to verify the result, after testing 8 kinds of pooled poultry environmental samples by PCR. The PCR detection limit of 1 CFU/mL was determined using lysis of cells from pure cultures, and the testing time was less than 48 h. On-farm samples were collected from the 2 kinds of layer farms per province: one of which was the layer farm that held more than 50,000 layers, and the other type was less than 50,000. It showed that the present PCR method is simple, inexpensive and effective in screening SE from on-farm samples in large quantities. The survey result identified only 1 SE-positive farm that held large-scale layers. In this farm, 9 samples were found contaminated with SE out of 1512 in total from eight voluntarily investigated farms covering drinking nipple (3), eggsaver (1), air inlet (1), air (1), overshoe (1) and eggshell (2). In conclusion, slight environmental contamination of *Salmonella Enteritidis* was detected on layer farms in the Northern China using specific PCR method. And various areas within farms may lay the very foundation of propagation chain for SE.

Keywords: *Salmonella Enteritidis*, environment, PCR, layer farm, North China

S6- 0008 Measurement of environmental variables in a high density stacked-cage layer house in summer

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The environment variables (e.g., temperature, relative humidity, air velocity and CO₂ concentration) in high density stacked-cage layer house during summer seasons were determined in Hubei province from July 7 to August 17 in 2015. The monitored house had a dimension of 80.3 × 11.6 m (L × W) contained four cage rows and each cage row had six tiers. The tunnel ventilation system with 18 fans of 1.3 m diameter in the back wall, with 40.95 m² cooling pads in the front wall, two cooling pads of 59.43 m² in both longitudinal wall. A portable measuring platform was used in this experiment, which was equipped with a temperature/RH/CO₂ sensor and an air velocity sensor. The environment variables were measured horizontally and vertically of 24 measuring points in the high density stacked-cage layer house. These data was collected 6:00, 12:00 and 18:00, respectively. The results showed that, the temperature, relative humidity, air velocity and carbon dioxide concentration of different measuring points were different along horizontal and vertical direction. The temperature varied from 20.5°C to 31.2°C, the average temperature was 26.6°C in summer. However, the relative humidity was more than 80%, which was higher for laying production using evaporative cooling system to decrease indoor temperature in summer. The average air velocity was 0.49 m/s at the egg collection machine site, and that was 2.62 m/s at the end of cage row. The CO₂ concentration was lower than 1500 mg/m³, which was in the suitable range for layer production.

Keywords: thermal environment factors, CO₂ concentration, laying hens houses

S6- 0009 Growth performance and economic appraisal as influenced by different bedding materials in 3 commercial broiler strains during hot climatic conditions

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A study was conducted to evaluate the effect of four different bedding materials on the growth performance, carcass characteristics and economic efficiency of three commercial broiler strains (Ross 308, Cobb 500 and Arbor Acres). 2. Three hundred and sixty day old broiler chicks, 120 from each strain having uniform body weight were procured and randomly distributed into 36 replicates under CRD with 10 birds in each. The experimental birds were reared on 4 bedding materials (wood shaving, wheat straw, corn cob pulp and rice husk) and fed same diet ad-libitum up to the age of 5 weeks under Completely Randomized Design with 4 × 3 factorial arrangement of treatments (brooding sources × strains) 3. Weekly weight gain, FCR and mortality was recorded to evaluate the ultimate growth performance and economical appraisal in terms of running cost/kg live weight. 4. The results revealed that Cobb 500 reared on corn cob pulp (CP) exhibited significantly ($P < 0.05$) better body weight gain, FCR, Point spread (PS), European Production Efficiency Factor (EPEF), Growth performance index (GPI) and superior carcass characteristics leading to higher profit margin with better livability in comparison to other broiler strains reared on other bedding materials.

Keywords: broiler, bedding materials, strains, growth, economics

S6- 0010 Improving in house air quality and Muscovy duck performances by using slate floor with bio-bedding system

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This study was conducted to test the advantages of the novel slatted floor with bio-bedding (SFBB) system in duck house air quality and Muscovy duck production performances. A total of 6000 Muscovy ducks with 30 days of age were randomly and equally allocated into 3 types of duck houses, which were SFBB, conventional floor bio-bedding (CFBB) and slated floor (SF) system houses, respectively. House concentrations of gas, dust, airborne lipopolysaccharide (LPS) and microorganisms at 8:00, 14:00 and 20:00 o'clock every 10 days were measured, and Muscovy duck production performances were recorded. During the experiment period, NH₃, total aerobe, *Escherichia coli*, "*Salmonella* and *Shigella*" and LPS concentrations were all lower in SFBB than in BB or CFBB ($P < 0.05$). Duck daily gain, livability and feed efficiency were higher ($P < 0.05$) in SFBB than in CFBB, and the latter two parameters were also higher ($P < 0.05$) than in SF. Results of the experiment demonstrated that, compared with either CFBB or SF, SFBB system offered better in house air quality that helped to improve Muscovy duck health and growth performances.

Keywords: production systems, slatted floor with bio-bedding, air environment, Muscovy duck, growth performances

S6- 0011 Raising goose in house on biology bedding or on slated floor above biology bedding

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The traditional goose production in China relies on use of water surfaces that could cause considerable water pollution. Following implementation of government regulations of "Water protection actions" in recent years, raising geese completely in house stands out from traditional production systems. In this study, goose house air quality and goose production performances were investigated in the two housing systems that employ conventional floor bio-bedding (CFBB) or slated floor above bio-bedding (SFBB). Two houses each of 7 m wide and 45 m long were all laid with biology bedding, and one of the houses (SFBB) was constructed with a slated floor 1.0 m above the bedding. Both houses were equally divided into 12 experimental pens of 18 m² which was allocated with 27 day-old Yangzhou goslings. During the 70-day experiment, in house airborne pathogenic bacteria counts, dust particles, lipopolysaccharide (LPS) were routinely determined. Gosling weight, feed consumption, mortality were also recorded. The results showed that as the experiment proceeded, so increased airborne bacteria (total bacterial, *E. coli*, *S. aureus*) counts, air LPS contents and dust particles (PM 10 and PM 2.5), which all were significantly lower ($p < 0.05$) in SFBB than in CFBB house. Goslings on SFBB gained weight at the same rate as those on CFBB, but had lower mortality (4.13% vs 6.16%). In addition, no feather pecking was observed in both systems which indicated good welfare status. These results demonstrated that, under low stocking density of 1.5 bird/m², goose can be raised completely in house away from open waters, and SFBB system performs better than the conventional CFBB system by providing better in house air environment quality and goose health.

Keywords: house production system, conventional floor bio-bedding, slated floor above bio-bedding, air environmental quality, goose production performance

S6-0012 Influence of various alternative bedding materials on foot pad dermatitis (FPD) in broiler chickens raised in a built-up litter system

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Broilers in the United States are frequently raised on built-up litter systems, primarily bedded with pine wood chips (shavings) or sawdust. There is continuing interest in alternative bedding materials as pine products are often in short supply and prices rise accordingly. Alternative bedding materials must be inexpensive, available in large quantities without excessive hauling expenses and must take up and release moisture without any damaging affects to bird health or product quality. FPD is both an economic and animal welfare concern for those raising broilers. Litter management must be a top priority of growers when built-up litter systems are being used and FPD can escalate if litter conditions deteriorate. Three trials at Auburn University have examined the utility of various alternative bedding materials across multiple flocks. FPD was evaluated with each bedding material over time. Bedding sources were compared to pine shavings (PS) and included large pine shavings (LS), pine bark (PB), chopped straw (CS), cotton gin trash (CGT), sand (S), and gypsum (G). Each comparison was made across three consecutive flocks with one week down time between each flock. FPD was generally low in PS, PB, S and G, while CS, LS and CGT showed increased FPD associated with increased litter caking. Birds raised on G showed reduced FPD over PS and PB in the first flock (4.75 vs 14 and 17.5% incidence), although no differences were noted in subsequent flocks. Pine shavings sifted to create beddings of different sizes showed improvements in FPD with a smaller particle size seen in new or recycled bedding (38 and 27% vs 88% incidence). Results obtained with CS may have suffered due to larger particle size as compared to PS (0.040 bulk density vs 0.110 in PS). Alternative bedding materials can be used successfully under commercial conditions if litter caking, and the resulting FPD isn't substantial.

Keywords: bedding sources, foot pad dermatitis, broiler

S6-0013 Application of CFD to improve airflow velocity in bird occupied zone in a tunnel-ventilated laying-hen house

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It is common knowledge that air movement around laying-hens is everything to cool birds in warm days, but the air speed is higher near the ceiling than in bird occupied zone (BOZ) due to the fact that air will tend to take the path of least resistance. This part of ventilation doesn't significantly relief heat stress of birds and was not necessary by the birds. On the basis of field testing, the CFD (computational fluid dynamics) technology was used to improve the air speed in BOZ by installing several windbreaks under the ceiling in a tunnel-ventilated laying-hen house. The appropriate space distance (SD=4.5m, 6m, 9m, 12m, respectively) between windbreaks was studied according to the air turbulence and house static pressure. The results showed that the air speed in animal occupied zone was obviously increased and the area of lower ventilation near the air deflector was reduced significantly when the windbreak were installed under the ceiling, as the unnecessary ventilation over the animals was deflected smoothly to the birds-level. When the average velocity in aisle was 2.0~3.0 m/s, the SD between the windbreaks was better to be about 6m in the experimental house, at this moment, little air turbulence occurred before the windbreak and ventilation resistance was relatively small.

Keywords: pad cooling system, windbreak, airflow velocity, laying-hen house

S6-0016 Study on regulation method and device development of pad and fan cooling system

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The paper aims to study the control model, abrupt temperature drop problem and equipment development of pad and fan cooling system in confined poultry house in summer. The intensity and distribution of abrupt temperature drop in a typical commercial laying-hen house in north China were investigated by field test. The results of field test show that over half of the house was cooled by more than 5°C (the maximum reached 12.4°C) within 25 min after starting the cooling system under ON/OFF control model. In order to solve this problem, a multistage regulation method of pad and fan cooling system was introduced. The aim temperature was reached gradually through temperature and single-cooling range, aiming to ensure a relative long period for chickens to adopt cooling process. Water supply devices of current evaporative cooling system were redesigned, adding a special designed water repartition plate on the top of the original water supply pipe. The position of the water repartition plate was regulated for changing the number of available watering hole on the pipes, so four degrees of watered area were achieved as 1/8, 1/4, 1/2, and full of pad area, temperature gradual cooling was realized. The results of the lab test show that the gradually watering and cooling works well under multistage regulation, as well, the cooling efficiency of stage 1-4 was 15.84%, 30.53%, 56.67% and 83.70%, respectively. In order to operate the multistage regulation conveniently, the automatic control system was developed. The multistage regulation method and involved equipment are able to control the cooling speed and range, eliminate the abrupt temperature drop while benefit to improving environment control level in confined poultry house.

Keywords: pad and fan cooling system, abrupt temperature drop, multistage regulation, equipment development.

S6-0017 Efficacy of slightly acidic electrolyzed water for environmental decontamination of a disinfection room using an ultrasonic disinfection system

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The efficacy of aerosolized slightly acidic electrolyzed water (SAEW) against *S. enteritidis* on different surfaces was evaluated in a disinfection room using an ultrasonic disinfection system. Stainless steel, cotton cloth and rubber surfaces were inoculated with *S. enteritidis* and were subjected to SAEW fogging using an ultrasonic disinfection system sited at a distance of 2 m for 2, 4, 6, 8 and 10 min, and then left for a further hour in the disinfection room. *S. enteritidis* was cultured from these surfaces before and after each treatment, and evaluated for reduction in colony forming units. The bactericidal activity of SAEW increased with increasing available chlorine concentration (ACC) and treatment time. SAEW at ACC of 50 to 150 mg L⁻¹ significantly reduced the population of *S. enteritidis* on stainless steel, cotton cloth and rubber surfaces by 0.82 to 5.50 log₁₀ CFU cm⁻², 0.96 to 5.35 log₁₀ CFU cm⁻² and 1.10 to 2.19 log₁₀ CFU cm⁻², respectively, whereas fogging with deionized water resulted in a reduction of 0.12 to 0.29 log₁₀ CFU cm⁻². Treatment of SAEW at ACC of 100 to 150 mg L⁻¹ for 8 to 10 min decreased the population of *S. enteritidis* to undetectable levels, achieving reduction of 5.50 log₁₀ CFU cm⁻² on stainless steel and 5.35 log₁₀ CFU cm⁻² on cotton cloth surface. It was also found that the effect of SAEW increased with decreasing population of *S. enteritidis* inoculated on surfaces. Complete inactivation of *S. enteritidis* on stainless steel, cotton cloth and rubber surfaces were observed when 1 to 2 log₁₀ CFU cm⁻² of the pathogen on surfaces were treated with SAEW at ACC of 100 mg L⁻¹ for 4 min. Results indicate that aerosolized SAEW is effective for reducing pathogens on stainless steel, cotton cloth and rubber surfaces, and may potentially minimize pathogen contamination in the disinfection room.

Keywords: Slightly acidic electrolyzed water, environmental decontamination, *S. enteritidis*, ultrasonic disinfection system

S6- 0020 Analysis of the hygienogram information and hygienogram scores obtained after cleaning and disinfection of poultry houses in Belgium (Flanders) during the period 2007 to 2014

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Cleaning and disinfection (C&D) of poultry houses is essential to manage farm hygiene. Adequate performance of the different steps in cleaning and disinfection and the use of suitable products are keys to prevent and control zoonoses and animal diseases. Hygiene monitoring on total aerobic flora using agar contact plates at different locations of the poultry house are used to evaluate the proper execution of C&D and result in hygienogram scores between 0 and 5 (ranging from very good to very bad respectively). Scores and information of the so-called hygienograms of each poultry farm were recorded (n = 20422 - 2007 to 2014) and analysed. The outcome variables were: year, season, husbandry system (floor, barn and aviary, battery and furnished cage), production type (breeding, rearing, production poultry), use of cleaning product, disinfectant and its active components, disinfection time and temperature and disinfection responsible. Descriptive analysis showed a decreasing trend for the average hygienogram score over the years 2007 to 2014. Remarkable differences were found between the different husbandry systems; with barn/aviary system and floor housing having a better score compared to furnished cage and battery (0.62 and 0.85 versus 0.88 and 0.99, respectively). Scores of production housings were worse compared to pedigree breeding, breeding and rearing houses. Cleaning protocols using a cleaning product, gave better scores than cleaning protocols without a product. Differences in scores between groups of disinfectants (with certain active components) were found. Disinfection protocols using formol gave the best scores (0.53). In addition, disinfection protocols using 2 different disinfectants showed improved results compared to the use of 1 single disinfectant. Scores are negatively correlated with the disinfection temperature. Finally disinfection carried out by an external firm resulted in a better score (0.72) compared to disinfection done by the farmer (0.85).

Keywords: poultry farm, cleaning, disinfection, hygiene score, hygienogram

S6- 0021 On farm comparisons of different cleaning protocols and development of a reliable measurement system to evaluate cleaning and disinfection in broiler houses

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Cleaning and disinfection (C&D) of the stable environment is an important part of the biosecurity measures on the farm. An evaluation of the effectiveness of C&D protocols can help farmers to reduce the infection pressure on the farm. The objective of this study was to 1) select the most suitable sampling methods and parameters to evaluate and compare C&D in broiler houses and 2) evaluate differences in C&D protocols. Following different cleaning protocols were repeatedly carried out and compared: 1) overnight (8 h) soaking with cold water followed by cleaning with warm water and cleaning product (CP); 2) overnight soaking with cold water followed by cleaning with cold water and CP; 3) cleaning with warm water using CP and 4) cleaning with cold water using CP. Sampling was performed on following moments: before the onset of cleaning (BC); 24 hours after cleaning (AC); 24 hours after disinfection (AD). BC and AD agar contact plates (ACP) and swab samples were taken from various sampling points for enumeration of total aerobic flora, *Enterococcus* spp. and *E. coli* (index for *Salmonella*). After cleaning, swab samples and ATP swabs were taken and a visual score was also assigned for each sampling point. Results showed that agar contact plates (ACP) were found to be less suitable than swabs for enumeration and evaluation. In addition total aerobic flora and *Enterococcus* spp. seemed to be better hygiene indicators to evaluate C&D protocols than *E. coli*. ATP analyses gave more objective information about the level of hygiene compared to visual evaluations. Cleaning protocols which were preceded by an overnight soaking with water caused a higher bacterial reduction. Moreover, soaking of broiler houses leads to less water consumption and reduced working time during high pressure cleaning. No differences were found between using cold or warm water during cleaning. Drinking cups, drain holes, and floor cracks were identified as critical locations for C&D in broiler houses.

Keywords: cleaning, disinfection, microbiology, broiler houses

S6-0023 Evaluation of reproductive indices of the Nigerian local chicken bred in the Niger Delta region

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The study was carried out to evaluate the effect of crude oil production environment on the reproductive indices of the Nigerian normal-feathered local chicken reared in the Oil rich-Niger Delta region of Nigeria. The birds were raised under the backyard management system. A hundred and twenty adult birds comprising 60 males and 60 females from two different environmental locations, namely, Oil Producing communities (OPC) and Non-oil Producing communities (NPC) were used for the study. In Nigeria, the OPC locations are frequently marked with crude and refined oil spillage, and gas flaring. Data were analyzed (SAS, 2010) using a one-way analysis of variance in a completely randomized design. Results showed that cocks raised in the NPC environment had higher mean testicular weight (8.10 ± 0.50 g) than those from the OPC location (7.07 ± 0.50 g) even though they lacked statistical difference ($P > 0.05$). Female reproductive indices were significantly ($P < 0.05$) affected by environment, with female birds of the NPC having higher mean values than those of the OPC. For instance, between hens in the non-oil and those in oil-producing localities: ovary weights (15.12 ± 0.80 g vs 7.72 ± 0.80 g), oviduct length (28.46 ± 0.21 cm vs 18.36 ± 0.21 cm), oviduct weight (32.72 ± 0.32 g vs 16.31 ± 0.32 g) and abdominal fat (2.9 ± 0.57 g vs 13.60 ± 0.57 g), respectively, indicated superiority of the former over the later. The female birds raised in the oil producing communities were thus characterized by poorer reproductive potentials than those from the non-oil producing communities. The findings could imply that crude oil pollution affects the growth and morphological development of the reproductive organs of the domestic fowl, thereby bringing about poor egg production as well as reduced meat protein intake among rural settlers living in the oil producing communities.

Keywords: normal-feathered chickens, oil pollution, reproductive indices, backyard management

S6-0024 Microbial-mineral biopreparation – a novel litter additive for mitigation of odorous emission from poultry production

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The main purpose of the poultry buildings is to provide space, food, water, air, controlled environmental conditions (temperature, humidity) and protection against predators, infectious diseases, and adverse atmospheric factors, all contributing to animal welfare. Barn air quality and gaseous and particulate matter emissions to the outside atmosphere are important for workers and people living in the vicinity of poultry operations. Poultry manure is a source of odorous emissions of gases such as ammonia, hydrogen sulphide and volatile organic compounds (VOCs). Thus, the problem of improvement of microclimate (indoor air quality) conditions and gas emissions especially in relation to odorous compounds, is of high importance. The aim of the study was to evaluate effectiveness of innovative microbial-mineral litter additive (biopreparation, six strains of heterotrophic bacteria on mineral carriers) in mitigating emissions of NH_3 , H_2S and odorous VOCs. Two types of manure were used in a laboratory set-up. Control and treatment chambers (0.8 L each) were filled with a 0.5 kg of manure. The manure surface was powdered with 50 mL of the biopreparation. After deodorization process (96 h) exhaust air samples from each chamber were collected into Tedlar bags and concentrations of selected odorous compounds were measured on gas chromatography. All analyses were performed three times using three independent replicates. Concentrations of ammonia, hydrogen sulphide, trimethylamine, dimethylamine and isobutyric acid were reduced by 52/57%, 65/60%, 46/52%, 56/51% and 53/63% respectively for laying hens/broilers manure. Based on these promising results, further experiments at testing the effects of dose and time are needed simulating practical conditions at poultry farms. This research was financially supported by the National Centre for Research and Development grant no. PBS2/B8/14/2014 "Innovative biopreparation for poultry production premises".

Keywords: microbial biopreparation, manure, deodorization

S6-0025 Effects of LED light intensity on the performance, meat quality and blood properties of broiler chicks

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Lighting requirements of birds have to be considered to find out the optimal light intensity. Therefore, the present experiment was carried out to know the effects of the different light intensities on performance, meat quality and blood properties of broiler chicks. A total of 1200 1-day-old Cobb × Cobb broiler chicks were used in the experiment (4 replications, 50 chicks/pen), and lighting intensity was applied and switching as follows: 20→20→20 lux, 20→20→10 lux, 20→15→10 lux, 20→15→5 lux, 20→10→10 lux and 20→10→5 lux from 0-7d, 8-21d and 22-42 d respectively. Our results revealed that weight gain, feed intake and FCR were not influenced by altering light intensity, but numerically increased by decreasing light intensity from 20 to 10 lux at second weeks of age. Similarly, meat quality properties were not significantly influenced by the light intensity. In the same way, serum cholesterol, triglycerides, albumin, high density lipoprotein, alkaline aminotransferase and aspartate aminotransferase were not altered by the light intensity. Therefore, the present result suggested that, it is better to use 20 lux for the first week and then provide 10 lux to optimize performance without affecting meat and blood properties of broiler chicks.

Keywords: light intensity, performance, blood, meat and broiler chicks

S6-0026 Evaluation of egg production, egg quality and serum biochemical of White Leghorn birds under thermal stress

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Thermo-tolerance in chicken plays important role in tropical poultry production. Two groups of high (HT) and low thermo-tolerant (LT) birds were selected from a base population (n=254) based on their egg production during hot-dry (36-38°C and 40% relative humidity (RH) and hot-humid (32-34°C and 70% RH) environmental conditions. The HT and LT birds were placed in a psychrometric chamber and subjected to 37°C and 60% RH (THI-78) daily for 6 hours for a period of 14 days. The production performance, egg quality and serum biochemical of these birds (n=27) were evaluated. Data were subjected to one-way ANOVA and significant differences between means were found by Duncan multiple range test. Percent hen day egg production (HDEP) and daily feed consumption was significantly higher in HT birds than the LT birds, however no difference was found in their daily water intake. The surface body temperature recorded by infrared digital thermometer at comb, neck, back, shank and feet of HT and LT birds did not differ. There was no significant difference in the egg weight, shape index, albumen and yolk content, shell thickness between HT and LT group, however the albumen and yolk index was higher in LT birds and the yolk color and yolk cholesterol content was higher in HT birds. Serum glucose level did not differ between HT and LT birds, but apparently higher serum cholesterol and protein level was found in HT birds. There was no significant difference in the corticosterone level between the HT and LT birds before exposure to thermal stress, however after stress apparently higher corticosterone level was observed in LT birds. Based on the above study we can conclude that high thermo-tolerant birds lay more egg under thermal stress and there is an increase in yolk cholesterol content but decrease in overall quality of the eggs.

Keywords: thermotolerance, psychrometric chamber, egg production, egg quality, serum biochemical, WLH chicken

S6-0027 Role of combination LED light color on performance, blood properties and muscular fatty acid composition of broiler chicks

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Performance of broiler has been studied under the monochromatic light color but little is known about the effects of combination light on broiler performance. Therefore, this study was undertaken to know the effect of LED light color on the performance, blood properties of broiler chicks. After hatch, a total of 1200 Ross x Ross chicks were equally divided into 6 lighting treatment with 4 replications. The LED light treatments were 1) Yellow (Y, 600 nm), 2) Green (G, 560 nm), 3) Blue (B, 480 nm) 4) mixing of Yellow-Green (Y→G), 5) Yellow-Blue (Y→B) and 6) Control white (fluorescent lamps). The light schedule was 24L: 0D, and intensity was 0.1 watts/m² at birds head level. Birds were allowed free access to feed and water. At 3rd and 5th weeks of age, blood samples were taken by puncturing the wing vein and separated serum was stored at -70°C until analysis. At first week, weight gain was not influenced by the light treatment. During the 2-3 weeks, weight gain was increased significantly in monochromatic Y (659.2 g) treatment ($P < 0.05$). In the 4-5 weeks period, weight gain was significantly ($P < 0.05$) higher in Y→B combination lighting color than that of W treatment (1099.5 g). During the third weeks, both glucose and triglycerides (TG) were numerically decreased in Y→B light treatment. In similar, in fifth weeks, Y→B light treatment significantly decreased glucose, TG and cholesterol level in blood. On the other hand, both meat properties and bone mineral density were not influenced by the light treatments. In muscular fatty acid composition, though, the saturated and unsaturated fatty acid content were not influenced by the light treatment, but oleic acid (C18: 1) content was increased by the Y→B and erucic acid (C22: 1) was increased by the Y light than that of other light treatments ($P < 0.05$). In conclusion, these results indicated that Y→B light treatment enhanced the growth performance of broiler and decreased the glucose, TG and cholesterol level in blood.

Keywords: LED light combination, performance, blood properties and broiler chicks

S6-0028 Evaluation of EFG broiler optimizer to predict the optimal nutritional feed composition and appropriate feeding schedule to achieve greater broiler performance or profitability

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The EFG Broiler Optimizer software uses genetic, nutritional, environmental and management variables to predict broiler responses and calculates optimum inputs for each economic circumstance. The objective was to evaluate the effects of optimization targets on predictions made by the EFG software. The variables evaluated were ingredient price (IP) and chicken price (CP) at either Base, +10% and -10%, and optimization method (OM) to predict the best feeding program (FP), nutrient density and amino acid content according to optimization objectives (OB): minimum FCR, minimum cost/kg (CPKg), maximum margin over feed cost (MFC) and maximum margin/m² per annum (MMA). Data from 12 flocks of 160,000 chickens each, split in 8 houses/flock was used. Data input included feed composition, FP, daily environmental temperature, mortality, health plan, lighting program, ingredient prices, and meat prices. The response variables analyzed were digestible lysine (DLys), metabolizable energy (ME), FP (pre-starter, starter, grower), final BW, cumulative feed intake (FI), FCR, MFC, MMA and CPKg. Data was analyzed by ANOVA to determine differences among variables. IP affected ($P < 0.05$) the optimum ME of starter and DLys of starter and grower, BW on females, FI and FCR on males. The CP affected ($P < 0.05$) DLys of starter, FI and FCR for females. OM affected ($P < 0.05$) pre-starter and grower ME for females and ME pre-starter and starter for males and BW, FI and FCR for both. The OB changed ME in the 3 dietary phases for females and pre-starter and starter for males, DLys for pre-starter and starter for females and performance parameters for females and males. In general, the economic parameters (MFC, MMA and CPKg) were affected ($P < 0.05$) for all variables. In conclusion, the EFG Broiler Optimizer software was sensible to the changes in the different factors evaluated to determine the optimal nutritional density and/or optimum feeding schedule to improve the profitability of the broiler business.

Keywords: growth models, profitability, econometric software, management

S6- 0029 Effects of LED spectral combination on growth, immune function, carcass traits and welfare of AA broilers

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Light is a critical environmental factor for birds as it allows them to establish rhythmicity and synchronizes essential functions. Previous studies showed different light spectrums play different roles in regulating growth, behavior etc of the birds. The LED light has been widely used as an alternate of incandescent lamp in poultry industry because of its energy efficiency. LED can be designed to output different spectrum. Optimizing spectrum combination is therefore crucial for the application of LED. This study was performed to investigate the effects of four spectral combination LED, named: A (21% Blue+30% Green+24% Yellow+25% Red), B (35% Blue+35% Green+18% Yellow+12% Red), C (27% Blue+30% Green+22% Yellow+21% Red), and D (42% Blue+28% Green+18% Yellow+12% Red) on growth, immune function, carcass performance, and welfare of AA broilers. A total of 600 1-day-old broilers were used. Each treatment included 5 replicate groups with 30 chicks each. Feed and water were provided *ad libitum*. Light program was 20 lx for the first week, and 5 lx (14L:4D:2L:4D) till the end (6 wks of age). The results showed that broilers reared under C had significantly decreased average daily weight gain than A, B, and D at 4 to 6 wks by 14.45%, 13.76%, and 12.90%, respectively ($P < 0.05$). C had a tendency to decrease average daily weight gain of 1 to 6 wks ($P = 0.07$). C decreased the average daily feed intake of 4 to 6 wks and 1 to 6 wks compared to D ($P < 0.05$). A decreased FCR compared to D by 9.14% ($P < 0.05$). Antibody titers versus Newcastle disease in D was significantly lower than that in other groups ($P < 0.05$). An increase of gait score was observed in birds reared under A, C and D groups (by 44.97%, 26.32% and 33.33% respectively) compared to B ($P < 0.05$). Spectral combination had no effect on eyeball development or carcass trait ($P > 0.05$). It was concluded that A light source not only good for growth performance of growing period, but also increase the immune function and welfare of AA broilers.

Keywords: LED, AA broilers, spectral combination, growth performance, immune function, welfare, led, AA broilers, spectrum, growth performance, welfare, immune function

S6- 0030 Effect of combinations of blue and green monochromatic lights on pectoral muscle growth and meat quality of broilers

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Our previous study suggested that green light promotes broiler growth during the early stage [posthatching day (P) 0 to P26], and blue light enhances growth during the later stage (P27 to P49). The purpose of this study was to discuss whether the combinations of blue and green monochromatic lights at critical points between the early and later stages of growth improve the skeletal muscle growth and meat quality of broilers as compared with white light. A total of 240 male Arbor Acres broilers on P0 were reared under white (W), green (G), and blue light (B) by using light-emitting diode lamps at 15 ± 0.2 lx on the light schedule of 23L:1D (light off at 2300 h) from P0 to P26. On P26 at 2300 h, the birds of G transferred to B (G→B), and the birds of B transferred to G (B→G), respectively. The remaining birds of W still reared in the original illumination until P49 (W→W). The body weight, feed conversion ratio, area of pectoral muscle fibers, meat quality and plasma growth hormone were detected. We found that G→B and B→G showed a significant elevation in body weight, breast muscle weight, myofiber area and plasma growth hormone ($P < 0.05$), and a significant decrease in the feed conversion ratio than W→W ($P < 0.05$). Moreover, as compared with W→W, the pectoral muscles in G→B and B→G had higher shear force value, water-holding capacity, and collagen, whereas cooking loss was lower ($P < 0.05$). These results suggest that the combination of blue and green might increase the plasma growth hormone level and better improve the growth and pectoral meat quality in broilers.

Keywords: monochromatic light combination, pectoral muscle, meat quality, growth hormone, broiler

S6- 0031 Effect of monochromatic light on circadian rhythmic expression of clock genes in chick retina

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Compared with mammal, avian has superior visual function and is sensitive to the light. Previous studies in our lab have shown that monochromatic light can affect the growth, development, immune function and productive performance of broilers. It is well known that retina is one of the three circadian pacemakers in avian, which plays an important role in regulation of the circadian rhythms cooperated with hypothalamus and pineal. In order to investigate the molecular oscillatory mechanism of avian retina circadian clock, AA broilers were reared under the red (660 nm), green (560 nm), blue (480 nm) and white (400-760 nm) LED lights with a light period of 12 h daily (12L: 12D) and an illuminance of 15 ± 0.3 lux at the bird-head level. The expressions of clock genes (Bmal1, Bmal2, Clock, Cry 1, Cry2, Per2 and Per3) in retina were detected using the methods of RT-PCR. The results showed that the expressions of Bmal1, Bmal2, Cry 1, Cry2, Per2 and Per3 appeared significant daily variations, except for Clock, and Bmal1, Cry1, Per2 and Per3 presented circadian rhythmic expressions in the various monochromatic lights. As compared with white light, green light elevated the expression levels of positive clock genes (Bmal1, Bmal2 and Clock), and red light increased the expression levels of negative clock genes (Cry1, Cry2, Per2, and Per3). Meanwhile, blue and green lights elevated the mesor of Bmal1, increased its amplitude, advanced its phase. However, red light reduced mesor of Bmal1, decreased its amplitude and advanced its phase. Moreover, red light elevated mesors of Cry1 and Per3, delayed their phases. On the contrary, blue light reduced mesors of Cry1, Per2 and Per3, decreased their amplitudes. These results demonstrated that green light promote the periodic expression levels of the biological clock RNA by positive and negative feedback loop interactions, thereby regulating their physiological behavior and being consistent with the environment.

Keywords: circadian rhythm, clock gene, retina, monochromatic light, chick

S6- 0032 Effect of monochromatic light during the incubation on post-hatch pectoral muscle growth and satellite cell proliferation of broilers

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Artificial illumination has been widely used in modern poultry husbandry. Previous studies reported that monochromatic green light promote the growth and development of broilers. However, few studies had focused on the effect of monochromatic light during the incubation on growth of skeletal muscle. Thus, our study was aimed to discuss the influence of monochromatic light during incubation on growth of pectoral muscle and clarify the potential mechanism. A total of 1000 Arbor Acres fertile broiler eggs were exposed to green light (G-group), blue light (B-group), red light (R-group), white light (W-group), or darkness (D-group, control) throughout the incubation period upon hatching, then were raised in white light condition. Results showed that broilers in G-group had heavier body weight (1.8-25.2%), higher pectoral muscle index (1.8-47.6%) and bigger muscle fiber area (15.1-67.0%) than that of other groups during post-hatching day (P) 1 to P10. Both studies in vivo and in vitro showed the number (6.0-54.6%) and proliferative activity (in vitro: 2.6-64.7%; in vivo: 10.1-64.9%) of pectoral muscle satellite cells in G-group were higher than in the other groups. The level of plasma IGF-1 (7.0-47.2%) and the expression of skeletal muscle IGF-1R mRNA (5.5-32.1%) were higher in G-group than that of in other groups. In addition, the exogenous IGF-1 (50-200 ng/ml) increased the proliferative activity of satellite cells by 31.7-122.0% in a dose-dependent fashion in G-group. These results indicated that monochromatic green light promoted posthatch muscle growth and satellite cells proliferation during chick incubation through IGF-1 signaling.

Keywords: chicken embryo, IGF-1, in ovo, monochromatic light, satellite cell

S6-0033 A metal oxide semiconductor-based ammonia measurement system for use in poultry houses

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Regular ammonia monitoring is required to ensure poultry performance and meet welfare parameters. There are low-cost, portable ammonia measuring devices, but they have drawbacks that limit their adoption. Due to its low-cost, long life-time, and short response time, a metal oxide semiconductor (MOS) ammonia sensor could be suitable for measuring ammonia in poultry houses. The objective of this study was to develop and test a hand-held management grade ammonia measurement system for use in poultry houses. Three brands of MOS ammonia sensors were evaluated in the lab and the best-performing sensor was used for further development and testing. Since the MOS sensor is affected by humidity and temperature, relative humidity (RH) and temperature sensors were collocated with the MOS ammonia sensor. Outputs of these temperature and RH sensors were used to develop compensation equations that improved the ammonia sensor's performance. Using broiler litter exhaust gas as the ammonia source, using the boric acid scrubber as reference, the sensor system proved to be accurate (relative error of 7%) and precise (coefficient of variation of 7%) even when using a generic compensation equation. Under the test conditions, the sensor system was more accurate than a commercial electrochemical sensor and required less frequent purging. The response time of the system was ~1.5 min, the total mass was <1.4 kg while material cost was <\$430. Hence, in addition to being convenient, the system provided acceptably accurate and precise real-time measurements of ammonia, oxygen, RH, and temperature. Better quality RH and temperature sensors could further improve accuracy of the system while a diffusive design could reduce cost.

Keywords: ammonia sensor, broiler, welfare, performance

S6-0034 Charcoal may be an economic option in brooding chicks

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Poultry production in rural areas suffering from chick rearing with brooding in lack of electricity. To find an option charcoal was considered to be observed for use in absence of electricity. Thus the experiment was conducted with three hundred sixty (360) day old broiler chicks of Ross strain and compared performance and carcass characteristics between two brooding systems (charcoal and electric brooding). Experimental birds were fed ad-libitum with starter, grower and finisher diet consisting of 220, 210 and 200 g/kg CP and 12.56, 12.77 and 12.98 MJ metabolizable energy. Body weight, feed intake, mortality were recorded and FCR was determined for performance and cut up were measured for carcass characteristics. Data were analyzed in analyzing package using computer for ANOVA. Body weight was recorded higher 1301gm at 4 weeks of age under charcoal brooding. Body weight gain was higher in electric brooding at 4th and 5th wk, respectively. Feed intake was significantly different during 2nd and 3rd week of age. Feed conversion ratio was determined same and significantly lower value at 4th and 5th week of age under charcoal brooding. Mortality was higher (4.93 %) under charcoal brooding ($p < 0.05$). Percentage of blood was significantly different in male where female showed insignificant differences as well as feather. Dressing, cut up and abdominal fat percentage was different ($p < 0.05$) which was insignificantly different by sex. The production cost was 80 and 81 taka per kg of live broiler at 4 weeks of age and 101 and 103 taka at 5 weeks of age under charcoal and electric brooder, respectively. It may be concluded that productivity and cost of production would be better under charcoal brooding compared to electric brooding in the rural poultry rearing system.

Keywords: charcoal, electricity, brooding, broiler chicks

S6-0035 Effect of different cage tiers and housing zones on productive performance and egg quality of commercial layer

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For certain health benefits of commercial layers battery cage system is commonly used, these cages are fabricated in four, five or up to eight tiers. However, higher number of tiers may expose birds to stress and drop their performance. Present study evaluated the effect of different cage levels and housing zones on productive performance and egg quality traits of commercial layer (LOHMANN LSL-LITE). To this end, six hundred forty eight 17 week old pullets were arranged according to completely randomized design and divided into 12 treatment groups having 6 replicates of 9 birds each. Treatment consisted of 4 cage levels (upper, center, lower and bottom) and three housing zones (near cooling pads, middle and near fans). Birds reared at different housing zones showed significant differences regarding body weight, egg mass and production %, however, cage levels did not differ significantly. There was no influence of cage levels and housing zones on egg shell weight, shell thickness, yolk color, yolk index and Haugh unit. It was concluded that modern cage tier system of commercial layers did not affect negatively on the performance of the bird.

Keywords: commercial layer, productive performance, egg quality, cage levels, housing zones

S6-0036 Measurement and correlation analysis of overlapping cage-rearing environmental parameters in winter

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In order to analyze the difference of environmental quality parameters and their correlation. To further optimize the environmental quality scale in egg chicken. In this experiment, four closed, five corridors, eight layers stacked in fifty thousand herds of three hundred and sixty days old Roman powder layer housing for target. Determination of environmental quality parameters in different locations at low temperature environment in winter (temperature, relative humidity, velocity, CO₂ concentration, PM10, TSP, NH₃, light intensity). From the wet curtain side to fan side, the values of temperature, CO₂ concentration, velocity and PM10 increased gradually, relative humidity showed a trend of decline, the velocity was lowest close to wet curtain side in summer. The temperature, CO₂ concentration, velocity, ammonia, TSP, relative humidity and PM10 also increased gradually in winter, and PM10 at wet curtain side was significantly lower than other points ($P < 0.05$). From lower cage to higher cage, the temperature, the concentration of PM10, CO₂, the value of ammonia increased with cage layer increased gradually, relative humidity, velocity decreased. The temperature of wet curtain side was significantly lower than other points, the upper determine the relative humidity is lower than the lower points. Upper corridor low velocity measured in experiment, it is suggested that adjust the fan mode, ensure that the upper velocity. Inner temperature in the winter was carried out at 20°C above average, but different inner cage layer between uneven distribution of temperature, temperature between the lowest and highest value difference between 6 °C; The average CO₂ concentration is 2000 mg/m³ average relative humidity around 43%, the relative humidity is low; so the ventilation rate should be increased on the premise of guarantee the temperature.

Keywords: layer, environmental parameters, correlation, overlapping cage-rearing system

S6-0037 Design of online monitoring system for henhouse environment

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In order to realize the real-time online monitoring of environmental parameters of the henhouse, an online monitoring system with the local storage and remote transmission of measuring data is developed in this paper, including the data acquisition terminal, the server, the client of three parts. The data collection terminal includes air temperature, wind speed, relative humidity, CO₂, NH₃, H₂S, PM10 and light intensity sensors and data collector component. All sensors are collected to the data collector through RS-485 bus. The data collector uses ARM 32 bit Cortex-M3 CPU STM32F103VB as the core processor. GPRS wireless data sending module can realize the real-time transmission of environment data to the remote server. Combining with SD card data storage module and the real-time clock module, the environment data is recorded and stored. The Server based on SQL Server 2008, ASP.NET Web platform development, for the cloud server for receiving data storage and processing. The client include Web query the client and the Android client, the user can through the web page or a smart phone APP to realize henhouse environment data real-time query. The monitoring experiments are carried out in a laminated automatic control henhouse with 8 layers, which contains 5 million hens. The result shows that the air temperature, CO₂, NH₃ and H₂S meet the requirement of national environmental quality standard of livestock and poultry farm, but light intensity, relative humidity, wind speed and PM10 in the unreasonable range. The corresponding optimization measures are given. Practice shows that the system is suitable for the accurate monitoring of henhouse environment, which has broad application prospects in large-scale livestock precision farming. And more precise measurements and reasonable monitoring topology for laminated henhouse should be researched in further work.

Keywords: monitoring, networks, environmental engineering, embedded system, henhouse

S6-0038 Effects of free-range chicken on properties of vegetation and soil

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Free-range is common mode of chicken production in west China. The aim of this study is to investigate the effects of free-range broiler chicken on properties of vegetation and soil, one meadow covered an area of 2001 square meters were randomly divided into three groups of 667 square meters each, set the stocking density of group one as 40 broiler chickens, group two as 80 broiler chickens, and the group three keep standing idle. Free-range start from 5-week-age to 22-week-age, properties of vegetation and soil were detected pre- and post-free-range. The results showed that properties of vegetation and soil changed obviously post-free-range, vegetation properties such as cover degree, richness, diversity and biomass of group one and group two were lower than those of group three, and those of group two were lower than group one, the levels of soil properties such as moisture, organic matter, effective nitrogen, available phosphorus and available potassium had been raised remarkably, and pH was significantly decreased, the changes of group two were higher than those of group one, the soil available potassium content of group two reached 984.42 mg/kg, which are 3.34 times than that of group three. We conclude that excessive free-range will be produced great negative impact on ecological environment, regulation of reasonable stocking density is a positive measure to keeping the sustained development of free-range chicken production.

Keywords: free-range chicken, ecological environment, vegetation property, soil property

S6-0039 Effect of brooding temperature and initial body weight on broiler growth performance

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Broiler producers often ask what brooding temperature is optimal for improving broiler growth and productivity. The purpose of the present experiment was to investigate if the optimal brooding temperature differs for day old broiler chickens with different body weight and with different parent flock age. At the University of Aarhus, an experiment was conducted with 1344 day old Ross 308 broilers from parent flocks with an age of 28 or 50 weeks. The experiment had a 2x2x2 factorial design with 8 treatments (2 Parent flock ages x 2 Body weight groups x 2 Brooding temperatures). Each treatment was replicated 6 times resulting in 48 units / floor pens. Immediately after hatching, the chickens were transported to the stables and for each parent age, the birds were divided into 2 groups with a body weight under or above the mean. Each group was placed in one of 48 pens that were evenly distributed into 2 stables with a brooding temperature of either 33 or 37°C. All birds were fed standard broiler diets. Data were collected 7 and 34 days post placement and analyzed statistically with a mixed model in the SAS package. The highest brooding temperature reduced the body weight and the mortality on day 7 and improved the FCR of the birds at day 34. Moreover, at the end of the experiment chickens with a high starting weight had gained 65 - 106 g more weight than chickens with a low starting weight. It was concluded that a high brooding temperature can down-regulate the initial growth rate which broilers can compensate for later in the growth period. This reduces broiler mortality and feed consumption and improves productivity.

Keywords: broiler, brooding temperature, start weight

S6-0040 Effect of palm kernel meal containing probiotic in the diet on performances and fecal ammonia emission of local duck

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The objective of this experiment was conducted to study the effect of inclusion level of palm kernel meal (PKM) containing probiotic in the local duck diets on fecal-ammonia emission, and performances of local ducks. One hundred twenty day old duck (dod) of local duck were assigned in twenty- floor pens in a curtain- side housed. The level of 0%, 10%, 20% and 30% PKM containing probiotic were applied in the diets of local duck. Diets were isocaloric (3,000 kcal of ME/kg) and contained 20% crude protein (CP), and were provided ad libitum together with water for the 6- wk trial. Ammonia emissions were measured using Kitagawa, aspirating Gas Pump AP-20. Then, for each treatment-replication, 50 gr of fecal material were collected from the house of local ducks and placed in 400 ml beakers and covered with plastic wraps. The ammonia concentration, pH, and water content of feces were measured after incubation for twenty four and forty eight hours. Experimental design used was Complete Randomized Design (CRD) consisting of four treatments with five replications. The results indicated that the level 30% of PKM containing probiotic had increased the live weight, body weight gain, but had no effect on feed conversion of local ducks. Furthermore, the level 30% of PKM containing probiotic was very effective to reduce the fecal-ammonia emission for twenty four and forty eight hour of measurement. The conclusion that the use of PKM containing probiotic at level of up to 30% in the diets was no negative effect on the performances and was very effective in reducing of fecal ammonia of local ducks.

Keywords: ammonia, probiotic, prebiotic, PKM, local ducks

S8-0002 Diurnal pattern of lay and timing of artificial insemination in egg-type laying hens under intermittent lighting regime

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The diurnal pattern of lay and timing of artificial insemination (AI) were studied for egg-type laying hens of pre-parental flock (Hisex White-R) under intermittent lighting regime. The birds were allotted in 4 analogous groups (105 birds per group; cage housing from 17 to 70 weeks of age under the same conditions of management and nutrition); the earlier designed lighting scheme for all 4 groups was 1L:4D:4L:1D:4L:10D with the start (at 3 am. The diurnal patterns of lay were studied in 26, 46 and 66 weeks of age in control group 1. The AI procedures were conducted once in 5 days: in control group 1 since 12 am; in experimental groups 2, 3 and 4 since 9, 10 and 11 am, respectively. The most of daily laid eggs (70.1% on average) were laid during dark phases and only 29.9% during light phases; 82.3; 89.9; 93.4 and 94.0% of eggs were laid since 1st light switch (at 3 am) to 9; 10; 11 and 12 am, respectively. Mortality + culling levels from 17 to 70 weeks of age in groups 1-4 were 13.3; 16.2; 12.4 and 13.3%, respectively; live BW at 66 weeks of age 1795; 1748; 1796 and 1791 g; egg production per average hen 290.9; 286.4; 290.5 and 291.0 eggs; egg weight 61.7; 61.6; 61.9 and 61.8 g; FCR (kg of feed per 10 eggs) 1.24; 1.25; 1.24 and 1.23; yield of eggs suitable for incubation 87.1; 87.7; 86.7 and 87.0%; egg fertility 96.6; 93.7; 95.7 and 96.9%; hatchability 86.5; 85.9; 87.1 and 86.5%; hatch of A-grade chicks 83.6; 80.5; 83.4 and 83.8%, respectively. The results showed that under intermittent lighting scheme 1L:4D:4L:1D:4L:10D the AI procedure can be started as early as at 10 am. It will give 2 additional hours a workday for insemination without any deterioration of productivity in chicken and any considerable change in personnel schedules.

Keywords: laying hens, intermittent lighting, diurnal pattern of lay, timing of insemination, hatchability

S8-0003 Effect of housing system on meat quality in broiler production

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This study was conducted to determine chicken meat composition and fatty acid structures of liver, abdominal fat and breast muscle (*Musculus pectoralisprofundus*) in broiler reared by cage (CH) and floor (FH) housing systems. For this purpose, 15 broilers per replicate having stable live weight were selected in each system. Dry matter, crude protein, crude fat, crude ash and fatty acids analyses were taken in summer, autumn and winter seasons. It was found that dry matter and protein ratios of breast muscle were similar between groups ($P>0.05$). The fat ($P<0.05$) and ash ratios ($P<0.01$) were significantly higher in FH system. The poultry meat had higher ratio of polyunsaturated fatty acids (PUFA), especially n-6 fatty acids related with the diet consumed by chicken. Cage housing system caused to accumulation of myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0) and heneicosanoic (C21:0) fatty acids in the chicken that significantly raised total saturated fatty acid (SFA) ratios of abdominal fat and breast muscle ($P<0.01$). Proportions of total monounsaturated fatty acid (MUFA), total PUFA and PUFA/SFA of abdominal fat tissue were found higher in FH system ($P<0.05$). Proportions of omega-3 fatty acids (n-3), omega-6 fatty acids (n-6), n-6/n-3 and PUFA/SFA were similar between groups in liver and muscle tissues ($P>0.05$). Consequently, cage housing system used by broiler production was inclined to synthesis of saturated fatty acids, especially in fat tissue, and changed muscle composition and fatty acid structure of chicken meat.

Keywords: broiler, cage housing, floor housing, meat composition, fatty acids

S8- 0004 Meat sensory traits as affected by floor, cage and their exchange rearing system in broilers

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Farmers always try to improve broiler rearing systems to maximize the profit by improving bird health. Whereas, a consumer always desired higher eating quality from the meat. In the present study, different rearing systems were compared for their effect on meat sensory properties. Ninety-six birds were randomly picked from a flock maintained under Floor, Cage and two alternate rearing systems i.e. transferred to floor after 21 days in cages, and vice versa at 6th week. Each treatment was replicated six times and four birds from each replicate were used for study. Breast fillet from each carcass was roasted without salt and spice and a panel consisting of 12 persons evaluated the samples randomly on 9-points hedonic scale (1 “Dislike extremely” to 9 “Like Extremely”) for meat sensory traits (color, flavor, tenderness, juiciness, and overall acceptability). The data obtained were analyzed using ANOVA technique with the help of SAS 9.1. Results of the present study showed comparable meat sensory properties in cage, floor and alternate rearing systems. Transferring birds from cage to floor or vice versa has resulted in no deterioration in meat eating quality. Thus, it can be concluded from the present study that birds can be reared in either rearing system without deterioration in meat sensory quality and consumer acceptance.

Keywords: broiler, sensory traits, transfer system, cage

S8- 0005 Haematology and serum biochemical profile of layer chickens reared on deep litter system with or without access to grass or legume pasture under humid tropical climate

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There has been a growing interest on the effects of access to pasture on poultry health status. However, there is a paucity of data on the relative benefit of grass and legume pastures. An experiment was conducted to determine the effects of rearing systems {deep litter system (DL), deep litter with access to legumes (LP) or grass (GP) pastures} haematology and serum chemistry of ISA Brown layers. The study involved the use of two hundred and forty 12 weeks old pullets. The birds were reared until 60 weeks of age. Eighty birds were assigned to each treatment; each treatment had four replicates of 20 birds each. Blood samples (2.5ml) were collected from the wing vein of two birds per replicate and serum chemistry and haematological parameters were determined. The results showed that there were no significant differences between treatments in all the parameters considered at 18 weeks of age. At 24 weeks old, the percentage of heterophyl (HET) in DL and LP were similar but higher than that of GP. The ratio of H:L was higher ($P<0.05$) in DL than those of LP and GP while LP and GP were comparable. At week 38 of age, the percentage of PCV in the birds in LP and GP were similar but the birds in DL had significantly lower level than that of GP. In the early production phase, serum total protein of the birds in LP was similar to that of GP but higher ($P<0.05$) than that of DL. At the peak production phase (week 38), the total protein in GP and DL were similar but significantly lower than that of LP. The albumin level in LP was greater ($P<0.05$) than GP but similar to that of DL. In the late production phase, the total protein in LP was significantly higher than that of DL but similar to that of GP. It was concluded that rearing chickens in either grass or legume pasture did not have deleterious effects on the health of laying chickens but improved some parameters including blood protein and heterophyl/lymphocyte.

Keywords: rearing systems, stylosanthes, cynodon, serum chemistry, haematology, layer chicken.

S8- 0006 Modifying aviaries to reduce keel bone and foot pad disorders in laying hens

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Non-cage housing systems are potentially more superior for the welfare of laying hens than cage systems. The increased space allowance and access to litter, however, increase the risk of keel bone and foot pad disorders. Therefore, the influence of hybrid and the presence of ramps on these disorders and on egg production was evaluated in small scale aviaries. At 17 weeks, 200 hens of 2 hybrids (Dekalb White (DW) and ISA Brown (IB)) were distributed across 16 pens. Half of the aviaries were equipped with ramps the other half without ramps. If ramps were provided, hens could negotiate heights for access to facilities without having to jump or fly. From 20 to 52 weeks of age, the number of first quality and floor eggs were recorded. Keel bone injuries and foot health were measured repeatedly between 17 and 52 weeks of age. Visual assessment of hyperkeratosis, foot pad dermatitis and bumble foot were performed as indication for foot pad health. The effect of hybrid and ramps on the incidence of floor eggs and production of first quality eggs was not consistent over time. Providing ramps reduced foot pad dermatitis ($P<0.001$), bumble foot ($P=0.004$), prevalence of keel bone fractures ($P<0.001$) and deviations ($P<0.001$) at all analysed ages. The hybrid effect on foot pad and keel bone disorders demonstrates genetic predisposition for the respective disorders. IB were more susceptible to hyperkeratosis ($P<0.001$) and keel bone fractures ($P=0.025$) but less to dermatitis, bumble foot and keel bone deviations (all $P<0.001$) compared to DW. Ramps had a greater effect on dermatitis in DW compared to IB ($P=0.003$). These results suggest that providing ramps is promising for reducing keel bone as well as foot pad disorders in non-cage systems for laying hens. The demonstrated hybrid effects on the investigated disorders offer opportunities to improve laying hen welfare by selective breeding for favourable traits. Further testing in commercial aviaries seems warranted.

Keywords: laying hens, keel bone, foot pad disorders, aviary, ramps

S8- 0007 Effect of cage tier level on growth performance of Japanese quail

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Present study evaluated effect of cage tier level on growth performance of Japanese quail at Avian Research and Training Centre, University of Veterinary and Animal Sciences, Lahore- Pakistan. A total of 300 day old Japanese quail were arranged into 5 treatment group according to completely randomized design having 3 replicates of 20 birds each. Treatment consisted 5 cage tier levels in conventional battery cage system. Effect of tier level on growth performance were evaluated for four weeks of age. Growth performance of Japanese quail did not differ significantly among different tier levels regarding body weight, weight gain, and times of gain, FCR and mortality. However, feed intake of Japanese quail in different tier levels showed significant difference. It is concluded that conventional battery cage system is good choice for rearing Japanese quail having no adverse effect on growth.

Keywords: tier level, growth, battery cages, tier level

S8- 0008 Effects of different raising systems on growth performance, carcass, and meat quality of medium-growing chickens

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A growing awareness of human health, nutrition, and animal welfare concerns has led to development of specialty markets for organic food. Birds produced in alternative system, such as free-range, are part of this trend. In this study we evaluate the impact of three raising system: indoor-floor (IF), cage (CA) and free-range (FR) on growth performance, carcass and meat quality. Three hundred and sixty 29 d Lingnanhuang female chicks were randomly assigned to 3 raising systems with 3 replicates. All birds were offered same diets and slaughtered on 90 d. Feed intake of birds in CA group was significantly higher than that in FR and IF groups ($P<0.05$) and FR group was the lowest. Birds in CA group gained more body weight than those in FR group ($P<0.05$) and had a better feed/gain ratio than other groups ($P<0.05$), because of the inherent variability in FR raising system. Eviscerated carcass percentage of birds in IF group was significantly greater than that in CA group ($P<0.05$). Abdominal fat yield of birds in FR system was significantly lower than other groups ($P<0.05$), due to the environmental condition in the outdoor paddock. There was no difference in pH, water-holding capacity, intramuscular fat, and inosine monophosphate content among systems ($P>0.05$). In conclusion, for medium-growing chickens FR raising system had significant negative effect growth performance, reduce fat deposition but consistent with high meat quality demand and animal welfare.

Keywords: raising system, growth performance, carcass, meat quality

S8- 0009 Environmental assessment of medium-growing broilers with three raising systems

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Aerial pollutant of animal growth environment is generally considered to be an environmental stressor, which produced during the animal growing and the decomposition of manure and impact animal growth and health. The objective of this study was to assess the impact of raising system: indoor-floor (IF), cage (CA) and free-range (FR) on harmful gas (NH_3 , H_2S , SO_2 and NO_2), and PM10 of medium-growing broiler chicken. Three hundred and sixty 29 d Lingnanhuang female chicks were randomly assigned to 3 raising systems with 3 replicates. Contaminants detected randomly at 10 A.M, lasting one week. Concentration of NH_3 and H_2S in the CA group was significantly higher than that of other groups ($P<0.01$), moreover, the FR group was lowest. Concentration of SO_2 in the CA group was significantly lower than that in the IF group ($P<0.05$), while the concentration of NO_2 in the FR group was significantly lower than that in the IF group ($P<0.05$). Concentration of PM10 was unaffected by the raising system ($P>0.05$). High concentration of NH_3 effecting growth rate, reduce egg production, injury the respiratory tract, increase susceptibility to Newcastle disease virus (NDV) and effect egg quality of birds. Similarly, H_2S , SO_2 , NO_2 are irritation bird nervous, respiratory system as well as mucosa of variety organs severely. In present study indicated that the FR raising system is an alternative system which has good living condition, improving birds comfort and welfare.

Keywords: environmental assessment, raising system, aerial pollutant

S8- 0010 Meat sensory and antioxidant status of broilers were affected by different production systems

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Purchasers are increasingly interested in products from special system, such as free-range, because they thought birds reared in naturally system could improve its growth condition and decrease stress. Furthermore, reports have suggested that birds reared in free-range production system provide superior nutrition, flavor and meat quality. In this study impact of production system: indoor-floor (IF), cage (CA) and free-range (FR) on meat sensory and antioxidant status of Lingnanhuang medium-growing broilers was evaluated. Five birds in each raising system slaughtered at 90 d, left side of pectoralis major muscle was selected for meat sensory and TOSC assay. No difference among raising systems of tasting and tenderness. Interestingly, smelling in FR raising system was lower than that in other two systems ($P < 0.05$), while enormous difference of flavor between FR and IF raising system was observed ($P < 0.05$). Flavor is thought to be the most important organoleptic factor determining meat sensory and purchasing decision of consumers. More exercises contribute to flavor, because inosine monophosphate and hypoxanthine are breakdown products of adenosine triphosphate and enhance flavor. FR raised birds muscle obtain the best TOSC level and significantly higher than that in other two raising systems ($P < 0.01$). FR raising system is an alternative production strategy that increase flavor, improve antioxidant status and appeal to purchasers in certain markets for high meat quality demand.

Keywords: meat sensory, antioxidant status, production systems

S8- 0011 Dust treatment in aviary systems

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Aviary systems offer laying hens freedom of movement and a litter area to perform scratching behavior. These system characteristics can influence dust concentrations in the poultry house and consequently have an impact on the working conditions of the farmer. To reduce dust in aviary systems, the Experimental Poultry Centre evaluated different measures: monthly removal of litter in combination with an ionization technique, monthly removal of litter, removal of litter every two months and a control group where no dust reduction measures were taken. The study was conducted in 4 climate independent aviary compartments housing 1860 hens during two different laying cycles (Lohmann Brown hens of 69 weeks old and Dekalb White hens of 29 weeks of age). Dust concentrations of different fractions (inhalable dust, PM₁₀, PM_{2.5} and PM₁) were measured twice during the trial period, using a portable measurement technique based on a light scattering principle. Different fractions could not be measured simultaneously. The results of the different treatment groups were expressed relatively to the dust concentrations of the control group (100 %). The compartments housing white hens of 29 weeks old showed the lowest relative dust concentrations for each dust fraction when litter was monthly removed in combination with an ionization technique. Compartments containing brown hens of 69 weeks old showed the best relative results for the dust fractions PM₁₀ and PM_{2.5} when litter was monthly removed, while the combination of monthly litter removal and an ionization technique scored the best relative results for the dust fractions inhalable dust and PM₁. These results suggest the possibility to reduce dust concentrations in aviary systems by using an integrated approach of measures conducted by the farmer (removal of litter) and an ionization technique.

Keywords: dust treatment, ionization, working conditions

S8- 0012 The profitability of broiler production in environment controlled houses in tropics

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This study was planned to assess the profitability of broiler farms operated in environmental Control Houses (ECH) in tropics. Price of purchase chicks, cost of labor, cost of feed, feed conversion ratio, mortality percentage, machinery depreciation, electricity, cooling and heating charges, rent and cost of building on the profit of the broiler producer were investigated. Data was collected from 35 broiler flocks housed in environment control houses. Data were collected through formal interviews. The average flock size was 30000 birds and average cost of day old chick amounted to Rs 45 which accounted 33.30% of total cost. Cost of finisher feed (Rs 53.50) established the maximum share of the total cost, accounting for 44.10% while cost of starter feed contributed 10.21% of total cost. Cost of vaccination and medication was 3.11% while the cost of labor accounted for 4.00% of total cost. Further stuffs such as repair cost, fuel, electricity bills, feeders, drinkers and litter cost contributed < 2% to total cost of production. The average net return gained by the farmers were Rs. 65000 per flock of 30000 birds about 50% of the farmers experienced either loss or were neither in loss nor in profit. Economic indicators values viz. Profitability index (PI) 00.30, Capital turn over (CTO) 01.50 Rate of returns on investment (%) 31.88 Rate of return on variable cost (%) 135.36 Rate of return on fixed Cost (%) 423.71 With a CTO of about 1.50 and PI of about 0.30, enhancements in broiler production is possible to increase the profits of broiler farmers in tropics

Keywords: profitability, broiler production, environment controlled houses, tropics

S8- 0013 Assessment of problems faced by broiler farmers in environment controlled houses in tropics

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Objective of the study was to assess the problems faced by broiler farmers. Data were collected by visiting 35 broiler flocks which raised in environment control houses. A questionnaire was developed to collect required information and data were collected through formal interviews. In the study area average flock size was 30000 birds. Major problems informed by broiler farmers were lack of basic skills regarding broiler farming, unavailability of labour, inaccessibility of inputs and harsh weather. Broiler production is a moneymaking business in the study area. With a Profitability Index (PI) of about 0.30 and Capital Turnover (CTO) of about 1.50, developments in the broiler production is likely to increase the profits to investment of broiler farmers. Absence of feed quality testing laboratory, variable feed prices and disease diagnosis facilities were major issues in the study area. Poultry farmers had to take the diseased birds to local veterinarians and the often guess at the diseases based on earlier practice and post mortem. Proper health and disease management may increase the productivity of broiler flocks which in turn increase farmer income. Absence of training biosecurity and disease control were the major reason for higher mortality in broilers. Higher mortality rates were due poor management, unsuccessful health attention programs and severe outbreaks of diseases. Antibiotic therapy, effective vaccination against diseases, and hygiene could decrease rate of mortality. It was therefore demanded that extension staff should be able to encourage farmers to convey about preferred changes in the poultry farming design and to accept suggested farming practices. The known disease as well as management problems in broiler production faced by the farmers should be lectured by the extension workers, research and development organizations.

Keywords: problems, broiler production, environment controlled houses, tropics

S8- 0014 Housing: plastered versus un- plastered brooder walls in poultry production

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An investigation was carried out to study the health impact of inadequate brooder house with poor make-up on chicks. The two brooder houses used were constructed with a mixture of sand and cementitious materials consisting of lime and/or gypsum to form concrete blocks. Brooder 1 was plastered with cement (cemented) and served as control while brooder 2 was not plastered with cement (un-cemented) and served as the treatment. One hundred (100) day old broilers of mixed sexes and also 100 cockerels were used in the experiment that lasted for 28 days. Fifty broilers (50) and fifty (50) cockerels were randomly assigned to the plastered and also fifty broilers (50) and fifty (50) cockerels were assigned to the un- plastered brooder houses. The results obtained showed that broilers placed in plastered building suffered 6 mortalities out of 50 representing 12% and also that 4 cockerels representing 8% died in the cemented brooder. Results obtained in the un- plastered brooder walls showed that 31 broilers representing 62% died while 22 cockerels equivalent to 44% suffered mortality. The high incidence of mortalities in the un- plastered brooder house could be due to the ingesting of mixture of cement/sand particles used in moulding the blocks which became too toxic for the chicks to handle at their tender age. Conclusion was therefore reached that keeping in view with the hazards of cement, it is advisable therefore, to plaster the walls of the poultry houses to prevent the birds from pecking and swallowing cement particles.

Keywords: broilers, brooder, plastered, cockerels, un- plastered

S8-0017 The effect of maternal age, rearing and housing environments on egg traits and growth of leghorn chicks

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Commercial strain leghorn breeders are reared and housed in a variety of housing systems where they are exposed to various physiological and psychological stressors associated with housing type. The objective was to investigate epigenetic influences of maternal age, rearing experience and housing environment on egg traits, and progeny weight and growth. Two cohorts of LSL- Lite leghorns were reared in aviary (AV) or conventional battery cages (CC) from day 1, then housed in AV (n=2), CC (n=12) or large furnished cages (FC) (n=12) from 16 wks of age. Hens from each housing environment (n=96) were inseminated with pooled semen at three ages: Young (25 wks of age), Ideal (44 wks of age) and Old (68 wks of age). Each cohort had four replicates of progeny (7 males and 7 females), identically reared in furnished floor pens, from the five rearing X housing treatments: Trt1 (AV x AV), Trt2 (CC X CC), Trt3 (AV x CC), Trt4 (CC X FC), and Trt5 (AV X FC). Egg storage, incubation and hatch were controlled. Shell thickness (μm), shell (%), breaking force (kgf), and interior quality (HU) decreased ($P<0.0001$) with maternal age; with a concurrent increase ($P<0.0001$) in egg weight (g) and yolk (%). Hatch weight increased ($P<0.0001$) with maternal age, but Old chicks exhibited slower overall ($P<0.01$) growth rates than Young or Ideal. AV rearing increased egg weight ($P\leq 0.0454$) over CC. Rearing interacted with housing to affect egg HU, composition, and progeny growth. Eggs from Trt2 hens had lower ($P\leq 0.0145$) HU, with Trt4 also lower ($P\leq 0.0446$) than Trt5. Trt1 hens produced eggs with less ($P<0.0001$) yolk (%), and more ($P<0.0001$) albumin (%) content. Progeny from AV reared hens weighed more ($P=0.0056$) at hatch and maintained heavier ($P\leq 0.0417$) body weights to 35 d of age. Trt2 progeny had lower ($P<0.05$) body weights than Trt3 to 49 d of age, and Trt1 Progeny at all intervals. The rearing experience of hens interacted with housing and had life- long effects on their egg traits and progeny.

Keywords: leghorn, epigenetics, rearing, housing, egg traits

S7-0005 Bio-gas and electricity production from poultry waste in India

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Indian poultry industry is facing one of the major problem is disposal of waste from poultry farms. Traditional disposal of poultry waste results in air, soil and water pollution and subsequently health hazards in human as well as in poultry. The secondary data was obtained and analyzed to utilize the poultry litter and manure for the production of bio-gas and electricity in India. Bio-gas can be utilized for household, transport and particularly production of electricity. India is producing about 360 cores of broiler chicks and 22 cores of layers every year. An average of 9486.1 million tons of hatchery waste is also being produced from broiler and layer hatchery. The estimated bird litter production is 14.584 million kg per day, from this waste 1.69 million m³ of bio-gas can be produced per day which equivalent to 0.73 million kg of liquid petroleum gas per day. A total of 5 kg of poultry litter is required to produce 1 m³ of bio-gas. A total of 80 m³ of biogas can be produced from ton of poultry manure. Poultry farms in India can generate bio-gas of 2,440 million m³ with 729 million chicken. Approximately 2 kw electricity can be generated from 1 m³ of bio-gas and India can produce 270 MW electricity annually from poultry litter. The cost of production of bio-gas is 6-9 U.S. cents / kWh. It is more evident that waste from poultry farm can be utilized for production of valuable renewable energy source.

Keywords: bio-gas, poultry litter, manure, electricity

S7-0006 Production of energy from poultry waste in India: an overview

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The poultry industry is currently facing a number of environmental issues because of accumulation of large amount of wastes from poultry farms, slaughter houses and hatcheries. Currently, 23.54 million MT of poultry manure, 9486 MT of hatchery waste and 1.15 million MT of slaughter house wastes are produced in India every year. The above figures are bound to increase in the years to come owing to the fast growth rate witnessed by the Indian poultry industry. Accumulation of large amount of wastes from poultry farms slaughter houses and hatcheries may pose disposal and pollution problems. Nitrogen and phosphorus present in the poultry excreta cause degradation of surface water. Ammonia emitted through excreta into the atmosphere is significant aerial pollutant associated with poultry production. Poultry farms in India can generate biogas of 2442 million cubic meter with 729.21 million chicken. India can produce 303 MW annually from poultry litter. The bio-diesel industry is infancy in India. Diesel consumption in India is estimated at 83.58 million tonnes in 2016-17. Bio-diesel required for 20 per cent blending would be 16.72 million tones. It is the need of the hour to reduce their impact on the environment by adopting cost effective technologies for handling and utilizing them and in the process earn some more profit too. In this paper, a few such methods like recycling of wastes such as animal feed stuff, composting, vermin-composting, bio-gas generation and production of bio-fuel are discussed.

Keywords: poultry waste, biogas, bio-diesel

S7-0007 Growth performance, litter quality and leg disorders of broiler chickens raised on different densities of wood shaving as bedding materials

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Litter or bedding material is used in the poultry house to provide comfortable environmental condition. Good litter condition results in better production. The study was conducted with an aim to investigate the effect of litter density on growth, leg health and litter quality of broiler chickens from d1-42 days. Day-old male broiler chicks (n=500) were distributed randomly into four litter densities [T1 (5CM), T2(8CM), T3(11 CM) and T4(14CM)] treatment, each treatment had 5 replicates with 25 birds per replicate cage in a completely randomized design. Wood shaving was used as litter materials to a thickness of 5 cm, 8 cm, 11 cm and 14 cm on the floor of the pen. Birds were reared on the litter floor open-sided housing condition with ad libitum feed and water throughout the trial period. Starter diet was provided the birds from d1-21, and finisher diet was used for the rest of the trial period. All diets are in mash form. Data on feed intake, body weight, feed conversion ratio and mortality were measured weekly. Litter sample was collected on day 42 to assess N₂, dry matter (DM) and moisture level (%). Footpad dermatitis and hock burn incidences were measured on day 42 to assess leg quality of birds. Results demonstrated that litter thickness had no significant ($P>0.05$) effect on feed intake, body weight, feed conversion ratio and mortality of broilers from d1-42 days. Nitrogen, DM or moisture level (%) of litter were also unaffected ($P>0.05$) between treatment. Leg disorders such as hock burn and footpad dermatitis were influenced ($P<0.01$) by treatment. The incidences of hock burn and footpad dermatitis were increased ($P<0.01$) in T1(5CM) and T2(8CM) than other treatments. It can be inferred from our current study that, leg health may be deteriorated by using low density bedding material without affecting growth and litter quality of broiler chickens.

Keywords: growth, footpad dermatitis, hock burn, litter thickness and composition

S7-0008 Changes in nutrient status , gas production and microflora change during the decomposition of poultry manure

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In order to study the role of the microflora succession rules and the changes of the corresponding material relations in the litter. Poultry litter was mixed, in equal quantities by volume, with a mixture containing equal quantities (by volume) of sawdust and rice husk. To test the efficacy of bacteria in decomposing such poultry litter, one batch of it was treated with a single strain of *Bacillus*, another was treated with a consortium of bacteria comprising several species, and the third was kept as a control. Decomposition started sooner in the treated batches than in the control batch. The production of two undesirable gases, namely ammonia and hydrogen sulfide, decreased over time in the treated batches and later remained constant at 0.3 ppm for ammonia and 0.6 ppm for hydrogen sulfide. The results show that microbial diversity is reduced after pre-piled high temperature. Inoculant preparations can guide microbial proliferation and advance to start the fermentation warming in the accumulation of fermentation litter and fermentation applications early. Total nitrogen (N), total phosphorus (P), and total potassium (K) increased gradually over time, whereas the content of ammonium nitrogen and of organic matter decreased. Therefore, inoculation with bacteria can hasten the decomposition of poultry litter. Summary: *Bacillus* genus bacteria of inoculation guided microbial proliferation and advance to start the fermentation warming in the accumulation of fermentation litter and fermentation applications early, and that also have the good effect for the retention of nitrogen, phosphorus, potassium. Harmful gas NH₃, H₂S from the fermentation litter have significantly reduced, and maintained at 0.3 ppm, 0.6 ppm in the late. The genus *Bacillus* is the main advantage microflora. To the late, the proportion of *Bacillus* is reduced, and reaches a state of dynamic equilibrium with manure microflora.

Keywords: litter, poultry manure, inoculant preparation, microflora, *Bacillus*

S7-0010 Comparison of the degradation of microbial inoculants and enzyme preparation of doxycycline in the soil

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Veterinary antibiotics in livestock and poultry production in the use of antibiotics and into the body of livestock and poultry and its metabolites with the feces discharged into the environment, microorganisms will change because of the change of living environment. Antibiotics resistance refers to the microbes are not sensitive to antibiotics, antibiotics resistance and acquired resistance. Generally refers to the expression of microorganisms antibiotics resistance is generally controlled by antibiotic resistance genes, so as to obtain pressure of microbial resistance is reserved and reproduce, but also makes antibiotics resistant gene dissemination. Therefore, controlling the environment antibiotic content is the effective means to reduce the spread of antibiotics resistance. The degradation of antibiotics in general can be divided into biodegradable and non biodegradable. In order not to make the micro ecological environment of the steady destruction of microbial degradation of antibiotics has become a better choice. Born with degradation The microorganism is difficult to be found in the natural environment, so the research about cultivating the ability of many antibiotics degradation microorganisms are also increasing, but the research on the mechanism of gene level of microorganisms on degradation of antibiotics were seldom mentioned. The use of microbial degradation of the environment but may promote the spread of antibiotic resistance genes. These problems need to get a clear answer. This paper will be screened doxycycline degrading microbial inoculants and made the crude enzyme, soil experimental analogy, in order to determine the possibility of doxycycline degrading bacteria harmful to the environment. To illustrate the microbiology of doxycycline resistance and degradation mechanism, and put forward the hypothesis of the two the contact, on the basis of the future use of enzyme and microorganism were analyzed and discussed.

Keywords: doxycycline, microorganism, enzyme, antibiotics resistance gene, degradation

S7- 0011 Optimizing nutrition of commercial poultry industry for minimal negative impact of poultry wastes on the environment through precision feed formulation

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The poultry industry has historically played a leading role among agricultural industries throughout the world. This leading role has led to the development of a highly specialized industry made up of more large, automated production and marketing units than any other livestock industry. Five major problems in commercial poultry production are: (1) pollution of environment with mineral in poultry faeces; (2) feeding excess nutrients; (3) over utilization of expensive feeds and low utilization of less expensive but more poorly digested feedstuffs; (4) increased feed intake with consequent nutrient wastage by poultry in alternative systems; and (5) increased nutrient requirements of imported new strains of poultry. Some of the tools to reduce animal waste include the following: (1) use of precise nutrient matrix; (2) use of precise ingredient matrix; (3) proper use of modifiers such as enzymes, microorganisms, antioxidants, mold inhibitors, friendly medications, and other by-products; (4) genetic improvements in animals and ingredients; (5) reduction of toxicants and antinutritional factors; dan (6) improved feed and ingredient processing techniques that will lead to better nutrient utilization. "Precision Nutrition" is a concept currently being introduced as a new approach to evaluate, adjust, properly utilize and possibly reduce the excretion of potentially damaging nutrients within livestock and poultry operations. The majority of early precision livestock farming development originated in Europe and the UK (c. 1990 - c. 1997), specifically at the Silsoe Research Institute, UK and Leuven University, Belgium. Further development has since taken place across the EU; Germany, Denmark, the Netherlands, Finland and the Volcani Research Centre, Israel, before spreading to Australia and the USA. Animal nutritionists therefore have a potentially large role to play in precisely reformulating diets to decrease waste to the environment.

Keywords: commercial poultry industry, precision feed formulation, environment

S7-0012 Safe disposal of dead animal carcass by co-composting with animal waste and their utilization as a new source of bio-organic fertilizer

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Dead animal carcasses and livestock and poultry manure contain a large amount of organic matter, and therefore can serve as a good substrate for the growth of beneficial microorganisms, such as *Bacillus lincheniformis*. The current situation of safe disposal of the dead animal carcasses and animal waste and their utilization as a new source of bio-fertilizer in Dalian district are reviewed in this paper. Animals raised in Dalian are mainly concentrated in Pulandian, Wafangdian and Zhuanghe, and the number of animals in the three districts account for 46%, 23% and 21%, respectively. A total of 6.6 million tons of animal waste were produced each year in Dalian, and wastes from pigs, cattle, poultry were 1.8, 2.5 and 3 million tons, respectively. According to a conservative estimate, the number of dead animals from pig, cattle, poultry was 190,000, 11,000, 9,000,000, respectively, plus the number of other dead animals, the total number will be over 10 million. These large number of dead animal and large amount of animal waste have the potential to be developed into a value-added organic fertilizer or even bio-organic fertilizer that are greatly needed by the ever-decreasing fertility of agriculture land. According to the new policy of "zero growth in the use of chemical fertilizers and pesticides by 2020", huge amount of organic or bio-organic fertilizer will be needed. Dalian produces the premium quality of various fruits and vegetables (blueberries, cherries, strawberries, cucumber, etc.). The high profit gained by these section of agriculture industry allow the producer to use more relatively more expensive fertilizer such as the bio-organic fertilizer. This paper aims at the approach and technology to turn the waste into treasure and improve the soil quality and meantime, the nutritional quality and sensational preferences of fruits and vegetables.

Keywords: animal wastes, dead animal carcasses, safe disposal, re-use of waste, microbial fertilizer (bio-organic fertilizer), fruits and vegetables

S7-0013 The resource utilization of chicken manure in livestock breeding industry

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The rapid development of livestock breeding industry is accompanied by a significant increase in the amount of livestock waste, especially chicken manure. Once the livestock waste are piled up optionally, the ecological environment and animal health will face severe challenges. Chicken manure is a kind of valuable resource that can be used in various fields. The resource utilization of manure are summarized as follows: First, livestock waste can generate energy. Studies have shown that the average low calorific value of chicken manure is 10.45 MJ/kg, so chicken manure is considered as a high quality raw material in power industry. Besides, chicken manure applies to product biogas. Microbe can be most effectively used manure to product biogas by anaerobic fermentation, which can achieve the purpose of protecting the ecological environment and resource recycling. Second, microbial organic fertilizer can be prepared by setting up chicken manure composting. Chemical fertilizers abuse destroy the structure of soil and debase the quality of crops. Microbial organic fertilizer can solve these problems. Microbe degrades macromolecule organic that difficult to exploit and converts it into beneficial ingredients for the crop growth, such as bacterial protein, humic acid, vitamin and amino acid. At the same time, pathogens can be killed during chicken manure composting process, achieving harmlessness of microbial organic fertilizer. Finally, chicken manure can be used as the raw materials to product feed. Since the chicken intestinal short and incomplete absorption, 70% of the ingested nutrients are not digested and absorbed completely, leading chicken manure contains a lot of nutrients, such as crude protein and vitamin B12. Therefore, the feed production is an important way for the resource utilization of chicken manure.

Keywords: chicken manure, biogas, microbial organic fertilizer, feed

S7-0014 The preparation of a rice blast –resisting biological organic fertilizer from swine manure

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Rice blast(*Phyricularia oryzae*)is one of the most common diseases of rice, which mainly using pesticides to control, but it cause drug residues, environmental pollution and a series of problems. This article intend to screening for anti *Phyricularia oryzae* dominant bacteria,then preparation of functional biological organic fertilizer, so as to achieve resource utilization and prevent rice blast. 15 dominant strains were isolated from the swine manure, among which 3 strains of dominant bacteria that had inhibitory effect on rice blast.By the sequence alignment and physiological and biochemical characteristic analysis, the results indicated that X-1, X-2 and X-3 were *Bacillus subtilis*,*Bacillus licheniformis*,and *Paenibacillus polymyxa*,respectively. We prepare anti rice blast composite microbial agents by using it. Set up static aerobic composting with swine manure and straw. After it was thoroughly mature the microbial antagonism agent of rice blast was inoculated (1ml bacteria agent/100g composting products) and biological organic fertilizer (BIO02) was obtained.Then the main quality index was measured(temperature,pH,C/N,E4/E6, water content),which can reach the standard of biological organic fertilizer technology in China (NY 884-2012). Then the pot experiment of rice indicated that the application of BIO02 could increase the indexes of rice's height, dry weight, chlorophyll, soluble reducing sugars and soluble proteins significantly, which indicated that BIO02 could promote the growth of rice. In the other pot experiment, the prevention rate of rice blast reached to 75.1% which was 40.3% higher than the control group of organic fertilizer.

Keywords: swine manure, rice blast, biological organic fertilizer

S7-0015 Effect of amoxicillin, ciprofloxacin and doxycycline from laying hens manure on methane production in the anaerobic digestion system

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Anaerobic digestion system is commonly used for livestock wastewater treatment. Different type of animal manure or antibiotics might have different effect on the methane production in this system. Because pig manure have been consisted with prior reports, this study used laying hen manure as model animal manure, amoxicillin (AMX), ciprofloxacin (CIP) and doxycycline (DOC) as model antibiotics to investigate the effect of antibiotic on methane production from anaerobic digestion system. Anaerobic digestions were conducted using laboratory scale digestion with effective working volume of 1300 mL. The temperature of anaerobic digestion was set at 35±1 °C . The experiment was set up as a control group, six experimental groups and each group has 3 replicates. The results showed that methane production of AMX groups, DOC groups had no significant different with control group. However, CIP groups were significantly inhibited the methane production compared with the control group, the inhibitory rate were 58.34% and 52.55%, respectively for the group with initial CIP concentration of 96.34 mg/kg and 40.43 mg/kg. At end of the experiment, AMX and CIP still can be detected. The VFA concentration of CIP groups were significantly higher than the other groups around 26.6%. During the experiment, AMX and DOC did not affected the pH, NH₄⁺-N,TN, organic matter and other indicators; CIP can significantly reduce the slurry pH and improve NH₄⁺-N , can affect the community structure of methanogens. Therefore, compared with AMX and DOC, CIP might affect the methanogens, changed the chemical environment of the anaerobic digestion system, then inhibited the methane production, it should be pay more attention in the laying hen farming.

Keywords: amoxicillin (AMX), ciprofloxacin (CIP), doxycycline (DOX), laying hens manure, anaerobic digestion

S7-0016 Methane and carbon dioxide emission in layer manure management system

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Animal husbandry has become one of the main sources of greenhouse gas emissions from agricultural activities and have been widely studied. As a very important part of animal husbandry, few study had been conducted on the gas emissions during animal manure management. This study choose scale layer farm in Hubei, China and investigate the methane (CH₄) and carbon dioxide (CO₂) emissions during four stages of animal manure management including cleaning, composting, transportation and recycling. The results showed that a 60 million layer farm produced 42080.58 L/d CH₄ and 850109.37 L/d CO₂ in manure management system. The belt-cleaning stage had 2715.33 ± 97.74 and 35729.87 ± 402.22 L/d CH₄ and CO₂ emissions; During composting stage, 7359.60 ± 99.76 L/d of CH₄ and 414243.96 ± 1383.64 L/d CO₂ were detected; And the farm produced 60 t/d organic fertilizer after composting and transported by truck to peach orchard for recycling. The CH₄ and CO₂ emissions was 5.65 ± 0.50 and 295.34 ± 12.99 L/d during transportation stage, and 32000.00 ± 560.22 and 399840.20 ± 1956.31 L/d during recycling stage had 76% and 47% of CH₄ and CO₂ emissions in manure management system. Every layer had 0.07 L CH₄ and 1.42 L CO₂ emissions into the environment during the manure management system. Although we only obtained first step result, the layer manure management system must be paid more attention on greenhouse gas emissions, especially recycling stage.

Keywords: layer manure, manure management system, recycling, CH₄, CO₂

S7-0017 In-vitro and in vivo standardization of poultry excreta for all-weather bio-gas production

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Alkaline pH, higher ammonical nitrogen content, lower fiber and C:N ratio are some of the reasons that make poultry excreta a poor substrate for bio-gas production. Furthermore, lower ambient temperature also reduces bio-gas production. Therefore, present study was undertaken to condition the poultry excreta for efficient bio-gas production in all-weather conditions. In-vitro standardization for efficient bio-gas production from poultry excreta was done through dilution (D), acidification (A) and carbonization (C) experiments. Best combination of 'D' and 'A' experiments was used for substrate formulation and in-vivo bio-gas production using 0.2 cum anaerobic digester and designated as T1. In T2, substrate was formulated using combinations of 'D', 'A' and 'C'. Diluted (D) poultry excreta without any other combinations acted as control (T0). The hydraulic retention time (HRT) was significantly lower (P<0.05) in T2 and T1 than T0. The pH was significantly higher (P<0.05) in T0 than T1 and T2. Total dissolved solid (TDS) and electrical conductivity were significantly higher (P<0.05) in T1 and T2 than T0. Bio-gas production was significantly higher (P<0.05) in T2 followed by T1 and was lowest in T0, both during summer and winter seasons. Qualitatively, blue flame proportion was more in T2 over T1, wherein, no flame was obtained in T0 group. It was concluded that conditioning of poultry excreta has better potential for all-weather bio-gas production.

Keywords: poultry excreta, bio-gas, dilution, acidification, carbonization, conditioning

S9-0001 Egg and poultry meat safety

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To meet the growing demands of consumers in Georgia on safe egg and poultry meat, from industry or farmers to consumers, not forgetting the feed manufacturers and poultry companies possible by improving health through nutrition, protection of safety of egg from salmonella and etc. Very important to follow up strict guidelines on hygiene and safety of egg and well known, that many antibiotics, when used at sub-therapeutic doses (much lower than the dose needed to treat disease), have also been found to improve the growth of livestock: they turn feed into meat, milk, or eggs much more cost-effectively. However, almost as soon as the growth-promoting properties of antibiotics in farm animals had become standard practice, reports began to appear in the scientific literature that showed that the bacteria were becoming immune to the drugs. There are a number of ways in which bacteria can develop immunity, but in the case of prophylactic antibiotics, it may be a case of "what doesn't kill you makes you stronger" When an antibiotic is used at a low dose, it might actually help make that bug immune. Food animals can become infected with *Salmonella* from feed and from the environment, so that many foods of animal origin, such as meat, poultry, eggs and raw milk can become contaminated. *Salmonella* may also contaminate fresh produce via contact with infected animals or other environmental sources.

Keywords: *Salmonella*, egg, antibiotics, poultry meat, georgia farmers industry

S9- 0002 Effect of beeswax coating on internal and sensory qualities of chicken eggs stored at room temperature

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Coating of eggs is one of the methods used to preserve egg quality and extend the shelf-life. Beeswax has film-forming properties and used as a coating material on fresh fruits and vegetables mainly to control desiccation. The objective of this research was to evaluate the suitability of beeswax as a coating material of eggs to control egg quality and sensory attributes over extended time duration. Total of 223 eggs (38-week old ISA-Shaver Brown) were purchased from a livestock farm. Eggs were individually weighed and assigned as completely randomized design to 03 different coating treatments: hot water extraction of beeswax, mineral oil (+ control) and uncoated (- control) and stored at room temperature. The weight loss, internal quality parameters (Haugh unit (HU), yolk index (YI), albumen and yolk pH, yolk colour) and sensory qualities of eggs stored at room temperature ($27 \pm 2^\circ\text{C}$) were determined. Microbiological analysis was done to identify the presence of *Salmonella* in eggs. Mineral oil and beeswax coated eggs had shown significantly ($P < 0.05$) low weight losses ($< 1.0\%$) throughout the storage periods compared to the control ($\approx 5.32\%$). The albumen pH of non-coated and mineral oil coated eggs considerably increased from 8.74 to 9.75 and 8.54, respectively, while beeswax coated eggs increased from 8.74 to 9.02 within 1 week then it decreased to 6.93 after 6 week. Based on HU, non-coated eggs changed from grade AA to C within 3 weeks. However, mineral oil maintained B grade of egg quality up to 5 weeks. Sensorial acceptance of beeswax coated eggs was lesser than that of non-coated eggs after 2 weeks of storage due to presence of black spots on egg white. All coated eggs were negative for *Salmonella* indicating that eggs were microbiologically safe throughout the storage. This study demonstrates that even though the weight loss is controlled with beeswax coatings, it cannot preserve the internal quality of eggs and extend the shelf life of eggs.

Keywords: beeswax, chicken egg, internal quality, sensory, shelf-life

S9- 0004 Comparison of nutrient composition and egg quality characteristics of Indian desi- chicken and commercial layer

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A study was conducted to compare the nutrient composition and sensory quality of Indian desi-chicken and commercial layer eggs. A total of 20 Indian desi-chicken and 20 commercial layer eggs were obtained from an organized poultry farm. Nutrient composition such as moisture, protein, energy, crude fiber, ash, calcium and phosphorus were analyzed as per standard protocol. The fatty acids composition of farm fresh eggs of Indian desi-chicken and commercial layer eggs were analyzed by gas chromatography using a fused silica capillary column and statistical analysis was done by using t-test. The sensory quality of egg was assessed by taste panel and results were recorded on a seven point hedonic scale and statistical analysis was done by using Kruskal-Wallis K-sample non-parametric test. Eggs from commercial layer showed significantly higher ($P<0.05$) moisture, protein, energy, ash, calcium and phosphorus than Indian desi-chicken. There was no significant difference in crude fiber content of eggs of commercial layer and Indian desi- chicken. Commercial layer eggs showed significantly ($P\leq 0.01$) higher omega-3-fatty acids content than Indian desi- chicken. Eggs from commercial layer showed significantly higher ($P<0.05$) egg weight and egg yolk colour. No significance difference was observed between shape, albumen and yolk indices and shell thickness of commercial layer and Indian desi- chicken. Sensory scores such as flavor and texture of egg did not show any significant difference between commercial layer and Indian desi-chicken. It is concluded that eggs from commercial layer had higher values of egg weight, moisture content, protein, energy, ash, calcium, phosphorus and omega-3- fatty acids than Indian desi-chicken, where as eggs from Indian desi-chicken had higher values of egg yolk colour and sensory scores such as colour and overall acceptability.

Keywords: nutrients, fatty acids, egg quality, Indian desi-chicken egg

S9-0006 Effect of tea polyphenols on production performance, egg quality and hepatic antioxidant status of laying hens in vanadium containing diets

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This study was conducted to determine the effect of tea polyphenols (TP) on production performance, egg quality and hepatic-antioxidant status of laying hens in vanadium containing diets. A total of 300 Lohman laying hens (67-wk-old) were used in a 1 plus 3 × 3 experiment design that hens were given the diet without vanadium and TP supplementation (control) or diets supplemented with 5, 10, 15 mg V/kg and TP(0, 600,1000 mg/kg) diets for 8 wks which included two phases, 5 wks accumulation phase and 3 wks depletion phase. During accumulation phase, dietary vanadium addition decreased (linear, $P<0.01$) albumen height and Haugh unit (HU), and TP supplementation mitigated (linear effect, $P<0.01$) this reduction effect induced by vanadium. Eggshell thickness (linear, $P<0.01$), redness (linear and quadratic, $P<0.05$) and yellowness (linear and quadratic, $P<0.05$) were decreased by vanadium and increased by effect of TP when vanadium-containing diet was fed. In depletion phase, the bleaching effect on eggshell induced by vanadium disappeared 1-wk after vanadium withdrawal. Eggshell thickness, eggshell strength, albumen height, HU were lower ($P<0.05$) in 15 mg/kg vanadium groups compared with control diet until 2-wk post vanadium challenge, but hens fed 15 mg/kg vanadium and 600 mg/kg TP groups shown no difference with control diet only after 1wk withdrawal. In liver, the activity of glutathione S- transferases was decreased (linear, $P<0.01$) and glutathione peroxidase was increased (linear, $P<0.01$) with the TP addition at 5 wk in accumulation phase in vanadium-containing diet, the malondialdehyde contents has increased (linear effect, $P=0.02$) with the addition of vanadium. The results indicate that supplementation of 10, 15 mg/kg vanadium resulted in reduced albumen quality, bleaching effect on eggshell color and antioxidant stress in liver. The effect of TP addition can prevent laying hens from the adverse effect of vanadium on egg quality and shorten the recovery time.

Keywords: vanadium, tea polyphenols, egg quality, depletion, laying hens

S9-0007 Lutein specific relationship among some spectrophotometric and colorimetric parameters of chicken egg yolk

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Lutein is dietary essential carotenoid required to prevent age related macular degeneration in human and responsible for the coloration of egg yolk. As the degree of colouration and lutein content of egg yolk are interrelated their degree of interrelationship calculated in this study. In view of the study wheat-barley based lutein free diet was offered to 28 pullets (Lohmann brawn, 20 weeks age) for a period of 21 days. Then 14 birds were fed diet containing marigold (80 mg lutein/kg feed) and other 14 birds offered feed containing oleoresin (45mg lutein/kg feed) followed by 21 days withdrawal of lutein. Eggs collected during trial was assessed for visual colour of yolk on a Petri dice using RYCF (0 to 15, where higher values of more colour) and Minolta Chroma Meter (a*-redness; b*-yellowness and L*-lightness). Yolk was analyzed for total carotenoids using a one stop device (iCheck[™]). HPLC method followed to determine the carotenoid components (lutein and zeaxanthin). Both trial showed that the increasing lutein enhance RYCF score (R2-0.87; P<0.01) as well as redness (R2-0.89; P<0.01). Total carotenoid has poor relationship with lightness (R2-0.13; P>0.05) and yellowness (R2-0.12; P>0.05) of the yolk. It may be concluded that the lutein is potentially responsible for increasing RYCF score and redness (a*) but not so much responsible for increasing yellowness (b*) and reducing lightness (L*) of egg yolk.

Keywords: carotenoid, HPLC, iCheck, spectrophotometry, yolk

S9-0008 Effect of feed inclusion of garlic and turmeric powder on laying hens' performance and egg quality

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This experiment was conducted to investigate, the effects of using various levels of garlic and tumeric rhizome powders, on production performance, on physical quality and the cholesterol content of the egg. A total of two hundred and sixteenth NOVOGEN White laying, 27- weeks of age, were divided into 9 treatments in a completely randomized design with 8 replicates and 3 birds in each replicate. Birds were fed a corn-soybean meal based (PF4) diet containing different concentrations of garlic powder (0.5%, 1%, 1.5%, 2%) and tumeric powder (0.5%, 1%, 1.5%, 2%) and control (0%). During seven weeks of experimental period, the data of production parameters were collected, some parameters of physical egg quality were followed and total cholesterol content in egg yolk was measured. Results indicated that the weight of hens, daily feed consumption and feed conversion were not affected by the diets supplemented with garlic and tumeric powder ($P \geq 0.237$). Mean values were respectively 116.5 (g/day) and 138.865 (g of feed/egg). No significant differences were detected, neither in egg production percent (84.76%), nor in egg-free rate of shell or even in egg size ($P \geq 0.189$). However, addition of 2% garlic powder reduced the rate of broken eggs ($P=0.0030$). The supplementation of garlic and tumeric powder had a significant effect ($P < 0.0001$) on the percentage of double yellow eggs. Albumen weight and diameter, and height and yellow index were also influenced by both phytobiotics' addition ($P \leq 0.0066$). Shell egg was heavier in tumeric 1% group, while it was thicker in garlic 1.5% hens. Feed inclusion of 1% garlic and tumeric powder tended also to decrease egg yolk cholesterol concentration ($P=0.087$). In conclusion, feed addition of garlic and turmeric powder can be used as alternatives to improve egg quality parameters.

Keywords: laying hens, garlic powder and tumeric, production, egg quality, cholesterol

S9-0009 Prevention of egg contamination by *Salmonella enteritidis* after oral vaccination of laying hens with *Salmonella enteritidis* defined mutants

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As up today eggs are still being identified as the main food vehicle causing human salmonellosis. In an attempt to dramatically reduce the risk of egg contamination caused by *Salmonella enterica* serovar Enteritidis, the *Salmonella* genome was screened for genes necessary for the survival of *Salmonella* Enteritidis in eggwhite. These essential genes were deleted and mutants were created. After extensive in vitro testing, several genes were selected and used as a vaccine in a 6 months in vivo trial. Two groups of 30 laying hens were vaccinated at day one, 6 weeks and 16 weeks of age with either one of the vaccine strains, while a third group was left unvaccinated. At 24 weeks of age, the birds were intravenously challenged with 0.5ml containing 5×10^7 cfu *Salmonella* Enteritidis PT4 S1400/94. Eggs were collected daily during 3 weeks before and after challenge and analyzed for the presence of the challenge and vaccine strain. Remarkably, this is the first trial to demonstrate that it is possible for a vaccine to completely prevent egg contamination. The successful development of these genetically defined vaccines is not only a major step forward but will pave the way for the development of a new era of vaccines.

Keywords: *Salmonella*, enteritidis, vaccine, egg, hen

S9-0010 Proteomic analysis of chicken, turkey, quail, pigeon, duck and goose egg albumen proteins revealed differential expression patterns

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Abundant bioactive molecules exist in poultry egg albumen, and are widely used in food processing, medicine, health care and other fields. However, except for chicken, the composition of egg albumen proteins in other main poultry species is unclear. To aid in unraveling diverse genetic and biological unknowns, a proteomic approach was used to analyze the egg albumen proteome of chicken, turkey, quail, pigeon, duck and goose, based on the isotopic versions of tandem mass tags (TMT) quantitative techniques. Three eggs per poultry species were collected for egg albumen proteins extraction and LC-MS/MS detection separately. The present analysis is the first to produce egg albumen proteomic data for most of the above-mentioned poultry species: a total of 161, 163, 169, 175, 140 and 151 proteins were identified for chicken, turkey, quail, pigeon, duck and goose egg albumen respectively. Lots of common and species-specific proteins were identified among species, such as 95 common proteins were both found in chicken (59.0%) and turkey (58.3%) egg albumen. Principal component analysis showed significant differences in proteomic patterns among these species, and the hierarchical clustering analysis generated three major sample clusters: (a) chicken and turkey (b) quail and pigeon and (c) duck and goose. The results can help revealing the quantitative egg albumen proteome pattern for main poultry species, which may provide potential directions for application of specific egg albumen protein production and will be meaningful for the investigation of species-specific biological components related to poultry egg albumen.

Keywords: TMT, proteomics, egg albumen protein, poultry species

S9-0012 Traits of eggshells and shell membranes of translucent eggs

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Translucent eggshells negatively affect the appearance of eggs and decrease their economic value. Causes of translucent eggshell formation have been investigated, but the primary reason is uncertain. In previous studies, scientists have found that the thickness of the eggshell membrane was significantly different between translucent and opaque eggs. However, there are some conflicts among studies. We performed 2 experiments with 3 breeding flocks of chickens to target the reasons for egg translucence. In experiment 1, eggs of 1,024 Brown-Egg Dwarf Layers (DWL) were used as experiment group. Approximately 1,600 eggs were collected over 2 consecutive days. They were stored for 3 days, and then 120 translucent and 120 opaque eggs were selected for measurement of egg quality traits and weight loss over several weeks. In experiment 2, we used DWL and White Leghorn pure line (WLL) for assessment of eggshell ultrastructure and membrane traits. We chose 120 translucent and 120 opaque eggs from 3,500 DWL eggs and 125 translucent and 125 opaque eggs from 5,028 WLL eggs. The results are as follows: (1) Translucent eggs had greater eggshell strength and lower shell membrane ultimate failure stress than opaque eggs in both DWL and WLL groups. (2) Translucent eggs had thicker eggshell and thinner shell membrane than opaque eggs in DWL. (3) No significant differences were found in either gas pore or bubble pore traits between translucent and opaque eggs in either line. (4) No significant differences were detected in internal egg quality or weight loss between translucent and opaque eggs was detected in either line. In summary, the present study suggests that variations in both eggshell and shell membrane structures are implicated in formation of translucent eggs.

Keywords: translucent eggshell, eggshell strength, shell membrane, ultimate failure stress, pore

S9-0013 The thickness of eggshell membrane exerted very weak effect on eggshell strength

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This study aimed at exploring the effect of eggshell membrane on eggshell strength. 900 hens from an F2 resource population constructed using White Leghorn and Dongxiang chickens were used as the experiment population. Eggs were collected at 66 weeks of age. Eggshell strength (kg/cm²) was determined using the eggshell force gauge (model- II Robotmation Co. Ltd., Tokyo, Japan) with the blunt end up. Shell slices with a size of 3 mm * 5 mm were taken in the equator of each eggshell and the membrane thickness were measured under the scanning electron microscope (JSM- 6301F). Descriptive statistics and correlation analyses were conducted with R project (R version 3.0.2). The results showed that the average thickness of eggshell membrane was 34.33±7.76 μm and the average eggshell strength was 3.166±0.930 kg/cm². The Pearson correlation coefficient between eggshell membrane thickness and eggshell strength was 0.0177 (P=0.6054). Although eggshell membrane was responsible for the initiation of eggshell mineralization and provided the platform for the whole process of eggshell formation, the result in this study showed very weak correlation between eggshell membrane thickness and eggshell strength. We suggested that the mineralization process after mammillae formation was independent to eggshell membrane. In a word, eggshell membrane thickness showed very weak effect on eggshell strength.

Keywords: eggshell membrane thickness, eggshell strength, correlation, chicken

S9- 0014 Correlation analysis of chicken eggshell brownness with egg quality

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Brown eggs are popular in many countries and consumers regard eggshell color as an important indicator of egg quality, eggs with dark brownness are perceived to be preferred by the consumers. Therefore, we carried out this study to explore if the degree of chicken eggshell brownness has correlation with other egg quality. Four pure lines laying brown eggs Y1 (n=2678), Y2 (n=2014), Y3 (n=3920) and Y7 (n=3492) from Beijing Huadu Yukou Poultry Industry Co. Ltd. were used in our study. At age of 36 week and 56 week, eggshell color, eggshell strength, eggshell thickness, egg shape index, egg weight, egg yolk color, albumen height and haugh unit were measured the day eggs were collected to analyse the correlation between eggshell brownness and other egg qualities. The eggshell color was measured with L*a*b* color system using reflectometer and the L value was used to represent the degree of eggshell brownness. L value denotes the reflectance values of eggshell and the darker the eggshell brownness, the smaller the L value. Results showed that at age of 36 week and 56 week, there was no discernible correlation-ship between eggshell brownness and egg shape index, egg weight, egg yolk color, albumen height and haugh unit for these four lines, but the eggshell brownness was significantly correlated with eggshell strength and eggshell thickness (-0.117~-0.401, $P<0.001$). Besides, when the eggs were divided into four groups according to the L value, the value of eggshell strength and eggshell thickness became greater with the darker of eggshell brownness. These results suggested that chicken eggshell brownness had no relationship with egg internal quality, but could reflect the eggshell strength and eggshell thickness in a degree.

Keywords: eggshell brownness, egg quality, correlation

S9-0015 Eggshell characteristics and cuticle deposition in three laying hens genotypes housed in enriched cages and on litter

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The objective of this study was to compare the eggshell characteristics and cuticle deposition of Lohmann Brown, Hy-Line Silver Brown and Isa Brown housed in enriched cages and on litter system. Our experiment was done in the second half of the laying cycle (40-56 weeks). The environmental conditions were kept the same in first phase of the laying cycle and according to laying hens requirements. Eggs were collected in four weeks interval to be 660 eggs in total. In our study, significant interactions of genotype and housing systems of eggshell quality measurements and cuticle deposition were not found. Significant effect of hen genotype ($P\leq 0.001$) was detected in egg weight and eggshell weight. The heaviest eggs were laid by Lohmann Brown (69.2 g in cages and 66.6 g on litter), while Hy-Line Silver Brown produced the lightest eggs (61.4 g in cages and 62.6 g on litter). Generally Isa Brown's eggs were significantly thicker ($P\leq 0.033$) than Hy-Line Silver Brown and Lohmann Brown. In spite of the significant effect of genotype on shell thickness the eggshell strength was not affected. The effect of housing system was observed in eggshell percentage ($P\leq 0.01$) and eggshell index ($P\leq 0.029$) with higher values on litter than in cages. The eggshell cuticle deposition was significantly ($P\leq 0.001$) affected by laying hens genotypes. Lohmann Brown produced eggs with significantly higher cuticle deposition (51.2 in cages and 50 on litter) compared to Isa Brown (45.3 in cages and 42.1 on litter) and Hy-Line Silver Brown (42.5 in cages and 40.5 on litter). The results of the current investigation pointed out the important effect of genotype on eggshell quality measurements and cuticle deposition in comparison with the lower effect of housing system.

Keywords: laying hen, housing, eggshell quality, cuticle

S9-0016 The effects of storage time on egg quality and yolk oxidation index

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The experiment aimed at revealing the change pattern of egg quality and yolk oxidation index over storage time. 510 brown-shell eggs and 300 blue-shell eggs were sampled and stored under the environment with the temperature of 24~27 °C and humidity of 68%~80%. Conjugated diene, malondialdehyde and surface hydrosulfuryl in egg yolk and regular quality traits of 50 eggs of both group in each time were tested every 7 days. Amino acids, fatty acids and cholesterol content in yolk of 30 eggs for both group were measured at the 1st day and the 70th day. Microbiological index of 10 brown-shell eggs in each time was detected at the 1st, 21th and 70th day. The results showed that the content of amino acids, saturated fatty acid and unsaturated amino acids in yolk had obvious reduction while there was no significant change in the cholesterol content in both group. The Haugh unit, the coefficient of egg yolk and the pH of egg white in brown-shell eggs changed faster in the prior 21 days than that in the late storage, and the pH of egg yolk increased rapidly in the prior 28 days. The Haugh unit of blue-shell eggs dropped fast in the first 14 days and the pH of egg yolk and white and the coefficient of egg yolk change fast in the prior 21 days. Bacterial colonies were detected in the egg yolk and white of brown-shell eggs after 21 days storage. The content of conjugated diene and malondialdehyde in egg yolk increased significantly over storage time, but the surface hydrosulfuryl dropped obviously. In a word, egg quality decreased and the degree of yolk oxidation increased over storage time, and the best preservation time was suggested to be no more than three weeks.

Keywords: storage time, egg quality, microorganism, oxidation

S9-0017 *Bacillus Subtilis* make a difference of the laying hens' production performance and egg quality during the late laying period

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During the late laying period, the absorption of hens decline, and the performance of laying decreases. The study was aimed to provide a basis for adding *Bacillus subtilis* to laying hen feed during the late laying period. Four thousands and two hundred, 427-day-old healthy laying hens (Hy-line white) with the similar weight and laying performance were randomly divided into two groups. Each group included three replicates, with 700 hens for each replicate. The control group was fed the basal diet, and the treatment group was fed the basal diet with *Bacillus subtilis* (100g/1000kg). *Bacillus subtilis* was developed and produced by the Shenyang Boshan Biological Technology Company. The pre-feeding period was 7 days, and the experimental period was 35 days. Because the decline of nutrition absorption due to the changing of metabolism during the late laying period, the laying rate reduces, the eggshell become thinner and fragile. The result shows that: the egg weight of treatment group exhibited significantly higher compared with the control group ($P<0.05$). The study during these years showed that: the production performance is improved probably because the *Bacillus subtilis* produce the Amino acids, Vitamin and other growth factors to enhance absorption and improve the feed efficiency. It needs more research of *Bacillus subtilis* to get more information. The study shows that compared with the control group, the eggshell color improve 5.27%, eggshell thickness improves 6.06%, the eggshell improves 10.11%, the proportion of yolk improves 5.2%, Haugh unit improves 7.8%, the treatment group exhibited significantly higher compared with the control group ($P<0.05$). Probably because the *Bacillus subtilis* produce the lactate and VFA to reduce the pH of intestinal, and the acidity environment promote the absorption of Ca and P. It needs more research of *Bacillus subtilis* to get more information.

Keywords: *Bacillus Subtilis*, Hy-line white hens, production performance, egg quality

S9– 0019 Metagenomic profiles of the gastrointestinal tract in chicken embryos

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The objective of this work was to identify microbial communities in the gastrointestinal tract of chicken embryos during the incubation period using T-RFLP (terminal restriction fragment length polymorphism) molecular genetic method. The study material represented samples of the gastrointestinal contents taken at the 16th day of incubation from chicken embryos, “Hisex white” and “Hisex brown” crosses. The composition of the gastrointestinal microbial community in chicken embryos was studied by T-RFLP method with CEQ 8000 (“Beckman Coulter”, USA). We received evidence that the microbiocenotic composition in the gastrointestinal tract of incubated 16-day old chicken embryos was characterized by rich taxonomic diversity. Thus, 30 phylotypes of various microorganisms were found in the embryonic intestinal microflora of “Hisex white” cross and 38 phylotypes- in “Hisex brown” cross. The gastrointestinal metagenomic community of “Hisex white” embryos was dominated (47.3%) by typical representatives of the avian intestinal microflora – microorganisms of family Enterobacteriaceae, representatives of orders Actinomycetales (13.6%), Bifidobacteriales (20.6%), family Lachnospiraceae (1.1%). The gastrointestinal metagenomic community of “Hisex brown” embryos was dominated (94.8%) by pathogens of order Rickettsiales. A number of unidentified bacterial genotypes were found in the embryonic digestive tracts of both crosses. These findings allow us to conclude that formation of a new microbiocological system in birds (the contents of the digestive tract together with the inhabiting microflora) takes place as early as during the embryonic development. This work is supported by RSCF (grant № 14- 16- 00140 «Modern views on the intestinal microflora of poultry in different rations: molecular genetic approaches»)

Keywords: intestinal microflora of chicken embryos, T-RFLP analysis

S9– 0020 Correlation between liver DNA methylation and egg yield of Hy-line brown laying hens

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In our country, the poultry industry is one of the highly intensive and socialized industries which have the most foreign exchange earnings, and chicken industry leads the main direction of poultry industry. Selection of egg type chicken is closely related to the whole poultry industry. With the increase of week-old hens, the egg production rate decreased, the short egg production cycle has restricted the development of the poultry industry. This study was to investigate the factors which had effect on egg yield from the DNA methylation level. With liver tissues among 56 week-old Hy-line brown laying hens between high-yield chicken (n=8, egg yield>90%) and low-yield chicken (n=8, 40%>egg yield>50%) population as the subject, the experiment adopted BSP-seq (Bisulfite Sequencing PCR) based on NGS to measure the Liver DNA methylation level on genome-wide scope. Then screened the differentially methylated genes related to egg yield and measured the levels of mRNA by realtime fluorescence quantitative polymerase chain reaction (RT-PCR). Finally, through adding folic acid in the fodder, differentially methylated genes related to egg yield were verified. As the results showed, possible differentially methylated genes related to egg yield were NME4, POLR1E, ATIC, PDE4B, PDE5A, ENTPD4, PRPS2, RPE, ALDOB, IL23R, MTMR3, MYO18A, OVOL2, STRN3 and NADSYN1.

Keywords: chicken, egg yield, liver, methylation

S9-0021 Abnormal egg shape effects hatchability in Pekin Ducks

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Hatchability and duckling quality are of the utmost importance for commercial hatcheries. There are many factors that can affect hatchability and quality of the newly hatched ducklings. Since poultry are hatched from an egg, the shell is crucial to the developing embryo. The importance of egg shell quality has been studied extensively in both turkey and chickens, however very little research has been directed toward ducks. This trial explored the effect that the overall shape of a duck egg plays on the moisture loss, hatchability, shell thickness, and pore concentration of eggs. Eggs were divided into normal shell and abnormal shell groups prior to incubation with 8 trays of 144 eggs per tray, per group. Abnormal shell is characterized by decidedly misshapen or faulty in texture with ridges, thin spots or rough areas. There were no significant differences in fertility due to egg shape. There was no significant difference observed in shell thickness, nor pore count between the contaminated, infertile, and early dead embryo containing eggs from either group. There was a significant difference ($P<0.006$) in the percent moisture loss, with the abnormal eggs losing more moisture than the control eggs. Significant differences ($P<0.0073$) were also observed in the average number of ducklings hatched by group. The control group hatched on average 7.23 more ducklings per tray than did the abnormal group. Similar results were observed in % hatched ($P<0.0073$) with a 5.03% difference in hatch rate between groups, as well as % fertile hatch ($P<0.0098$) having a 4.2% difference in hatch rate between the control and abnormal groups. Selection against abnormal egg shell in breeding programs could improve hatchery outputs and increase the efficiency of commercial duck production.

Keywords: hatchability, quality, ducklings, shell, abnormal

S9-0022 Effect of different levels of Purslane extract on performance, egg quality properties and morphology of the genital laying hens reared under high temperature conditions

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An experiment was conducted to determine the effects of Purslane alcoholic extract on performance, qualitative traits of egg and morphology of genital system of laying hens. One hundred and twenty Leghorn Hy-Line (W36) strain in 44 to 52 weeks of age used in a completely randomized design with 4 treatments and 5 replicates. Four experimental treatments were: 0, 0.1, 0.2 and 0.3 % of Purslane alcoholic extract which added to basal diet. During the experiment feed consumption, egg weight, egg production percent, egg mass and feed conversion ratio were measured weekly and periodically. At the end of experiment, one hen per replicate was slaughtered to determine morphology of genital system. The results indicated that there were no significant differences in performance parameters among treatments ($P>0.05$). Also, Haugh unit, shell thickness and egg yolk percent did not affected by experimental additives ($P>0.05$) but, supplementation of diets with Purslane extract did have increasing effect on strength of shell and percent of egg shell at the total of experiment ($P<0.05$). By adding Purslane extract to diet, albumin relative percent and yolk color was increased in total phase of experiment ($P<0.05$). In this study, adding Purslane extract led to significantly ($P<0.05$) increase in ovary and oviduct weight and number and weight of large yellow follicles compared to control group ($P<0.05$). The results under this experiment showed that Purslane extract adding into laying hens diet, can be promote qualitative traits of egg under high temperature environments.

Keywords: egg mass, feed consumption, follicle, leghorn, oviduct, purslane.

S9-0023 Reducing the prevalence of Salmonella- infected poultry flocks by a surveillance- and- elimination strategy-the Danish success

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In response to the rising incidence of human salmonellosis in Denmark during the 1990s, The National Danish Salmonella Control Programme for broiler and table egg production was implemented in 1996. The programme forms part of the Danish Parliament and Government's general objective of improving the quality and safety of Danish food. The programme is accomplished in collaboration between the national authorities, the poultry industry, and the farmers associations. Today 20 years later, the results of the programme are obvious. Since the launch of the programme, the prevalence of flocks infected with Salmonella have decreased by more than 20 % to less than 0,6 % in both the table egg and the broiler sector. In 2012, an EU Salmonella guarantee for the Danish table egg production was achieved. In 2015, no infected table egg flocks were found, even though each flock was tested by sock samples every second week. For the broiler production, all broiler flocks are tested twice during production. The number of infected broiler flocks is decreasing, and in 2015 the prevalence was below 0,6 %. The programme is a 'top-down' surveillance-and- elimination strategy, whereby infected flocks are eradicated by means of compulsory destruction or slaughter. There is a zero tolerance for all Salmonella types in all poultry products in Denmark. Vaccination has never been used in the programme. The sampling programme includes both serological and bacteriological analyses. The programme also comprises code of practice guidelines, import restrictions of animal material, biosecurity guidelines, requirement for heat-treated feed, and control by national officials. Conclusion: It is possible to reduce the prevalence of Salmonella- infected poultry flocks by surveillance and elimination strategies. The total cost of the programme is more than 15 mill. €. Since 2002 all the expenses have been paid by the producers themselves.

Keywords: Salmonella, elimination, quality, food safety.

S9-0024 Comparison of n-3 polyunsaturated fatty acid- enriched eggs from hens fed diets supplemented with microalgae and flaxseed

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Due to high content of eicosapentaenoic acid (EPA), microalgae, such as *Nannochloropsis oculata* (NO), becomes one of the important resource of n-3 PUFA and a potential alternative for flaxseed (FS) or fish oil. The purpose of this study was to compare quality of egg from laying hens fed diets supplemented with EPA-rich NO and FS by measuring n-3 PUFA content, yolk chemical composition, and sensory evaluation of boiled egg. A total of 216 healthy Lohmann Brown laying hens aged 37 weeks with similar laying rate and body weight ($P > 0.05$) were randomly allotted to 3 groups (6 replicates, 12 birds/replicate) and fed 3 experimental diets supplemented with none (CON), 8% NO (NOG), 8% FS (FSG) for 4 weeks. The diets were isoenergetic and isonitrogenous by varying the percentage of carbohydrate and protein sources. Feed and water were provided for ad libitum consumption. Data were analyzed by one-way ANOVA using SPSS 19.0 software package for Windows. Fatty acid content in yolk were different between diet NO and FS ($P < 0.001$). Total cholesterol, crude fat and crude protein were not different ($P > 0.05$). Docosahexaenoic acid (DHA) content in NOG group was 111.62 mg/egg, larger ($P < 0.05$) than FSG group (98.66 mg/egg), but total n-3 PUFA content of NOG (148.59 mg/egg) was lower than that of FSG (383.27 mg/egg). For sensory evaluation, scores from trained panelists for yolk fishy smell, hardness, and elasticity of egg white in NOG group were all higher ($P = 0.001$, $P = 0.014$ and $P < 0.001$, respectively) than other groups. More than half of panelists chose NOG egg as their favourites. In conclusion, *Nannochloropsis oculata* in laying hens diet enhanced yolk DHA and total n-3 PUFA content without change of other chemical compositions. Fishy smell and hardness were found in NOG yolk, but overall sensory quality was not influenced.

Keywords: *nannochloropsis oculata*; flaxseed; n-3 polyunsaturated fatty acid; docosahexaenoic acid; sensory evaluation

S9- 0025 Effect of dietary magnesium and limestone particle size on performance and eggshell quality of hens

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The aim of the study was to evaluate the effect of dietary magnesium (Mg) and grain- size of limestone on performance and egg quality parameters. A completely randomized experimental design with a 2 x 2 factorial arrangement of treatments was employed: 2 magnesium levels (1.56 and 4.0 g/kg) and 2 types of grain size of limestone (fine limestone (FL; <0.5 mm) and coarse limestone (CL; 0.8~2 mm)) in mixed feed. The concentration of Mg in the diet was increased by the addition of MgO. Coarse limestone significantly increased hen- day egg production ($P=0.043$) and Mg (in dietary concentration up to 4 g/kg) increased egg weight ($P<0.001$) and albumen percentage ($P=0.007$) and decreased feed conversion ratio ($P=0.049$). The addition of Mg to the mixed feed, together with CL, increased shell thickness ($P=0.014$), shell breaking strength ($P=0.003$) and shell percentage ($P=0.044$) and decreased yolk percentage ($P=0.008$). Fine limestone increased the concentration of Ca in the egg shell ($P<0.001$). In conclusion, the combination of Mg addition in form of MgO and CL in diet, with Ca:NPP ratio 12.8, positively influenced especially egg shell quality.

Keywords: magnesium oxide, limestone granularity, egg production, egg shell strength, hen

S9-0027 Effect of initial body weight and body composition of TETRA SL laying hens on the changes in their live weight, body fat content, egg production and egg composition during the first egg-laying period

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The experiment was carried out with 45 TETRA SL laying hens, which were divided into three groups based on their live weight on the one hand and based on their body fat content (determined by means of computer tomography) on the other hand measured both at 20 weeks of age. The average live weight of the hens in the “low live weight” group was 1534 ± 87 g ($n=14$), in the “medium live weight” group 1696 ± 33 g ($n=16$) and in the “high live weight” group 1861 ± 94 g ($n=15$). The average fat index calculated from the CT images was 22.9 ± 0.9 ($n=15$) in the “low fat” group, 24.9 ± 0.4 ($n=14$) in the “medium fat” group and 27.2 ± 1.6 ($n=16$) in the “high fat” group. The live weight of the experimental birds was recorded at 32, 52 and 72 weeks of age during the first egg-laying period. At the same ages the body fat content of the hens was also determined by means of computer tomography in vivo. Eggs, which were produced by the experimental birds one day before the CT examinations, were collected and, after breaking them, their yolk and albumen was separated, weighed and their ratio to the egg weight was calculated. The dry matter, crude protein and crude fat content of the eggs were analyzed chemically. Based on the results it was established that the initial body weight correlated with later measured body weight, the initial body fat content correlated also closely with body fat content measured at later stages of egg production. The low initial body weight and the high initial body fat content of the hens resulted in the lowest egg production intensity. Neither the initial body weight nor the initial body fat content of the hens affected the composition of their eggs significantly ($P>0.05$).

Keywords: laying hen, body weight, body composition, egg production, egg composition

S9-0028 Tea polyphenols alleviated the adverse effects of vanadium on gel quality, foaming property of egg white in laying hens

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Vanadium is one of the essential trace elements for human and animals, but excessive vanadium has been shown to decrease the egg albumen height (HU). Tea polyphenol (TP) is known as a strong antioxidant to improve the performance of animal. This study was conducted to try to alleviate the adverse effects of vanadium by addition of TP in laying hens. A total of 300 Lohman laying hens (67-wk-old) were used in a 1 plus 3 × 3 experiment design with a basal diet without or with vanadium or TP addition at V 5, 10, 15 mg/kg or TP 0, 600, 1000 mg/kg for 5 wks. The results showed that dietary vanadium addition decreased (quadratic, $P < 0.01$) the HU with time depended effect time ($P < 0.01$), and TP supplementation alleviated this reduction induced by vanadium. The hardness, gumminess, and chewiness of egg white gel (linear, $P < 0.05$), adhesiveness and resilience (quadratic, $P < 0.05$) were decreased by vanadium addition, while addition of TP increased the hardness, gumminess, chewiness (quadratic, $P < 0.01$), adhesiveness, and resilience (linear, $P < 0.05$) in vanadium-containing diet. There were no significant effects of vanadium or TP on egg white gel springiness and water binding capacity. And the vanadium in diet had no significant effects on egg white foaming properties and pH value (linear, $P > 0.05$). In conclusion, the dietary vanadium at 5, 10 and 15 mg/kg decreased the egg white quality (lower HU) with the negatively effects on egg white functional properties, while dietary TP at 600 or 1000 mg/kg alleviated the adverse effects.

Keywords: tea polyphenols, vanadium, egg quality

S9-0030 Effects of conjugated linoleic acid on the egg quality characteristics, n-3 polyunsaturated fatty acid and cholesterol content in egg yolks of laying quails

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The objective of this study was to investigate the effects of dietary CLA on the egg quality characteristics, n-3 fatty acid polyunsaturated and cholesterol content in egg yolks of quails. Four hundred 13-week-old laying quails were randomly assigned to 5 dietary treatments with 4 replicates. Laying quails were fed corn and soybean meal-based diets containing 0%, 0.75%, 1.5%, 2.25% or 3% conjugated linoleic acid (CLA) for 42 days, respectively. During the test period, eggs were collected to analysis on d 7, d 14, d 28 and d 42 respectively. The results showed that the average egg weight of 3% CLA group was significantly decreased after 14 days while it decreased after 28 days in both 1.5% and 2.25% CLA groups ($P < 0.05$). The yolk weight, egg-shaped index and shell thickness were not influenced by CLA. The cholesterol contents of yolks decreased significantly with increasing dietary CLA at d 7, but for 1.5% CLA group, it had best effect to lower cholesterol after 14 days ($P < 0.05$). Increasing the amount of CLA fed to quails would decrease the total n-3 polyunsaturated fatty acids (PUFA) content of yolk. Specifically, docosahexaenoic acid (DHA) content was significant decrease ($P < 0.05$) while eicosapentaenoic acid (EPA) and α -linolenic acid were no significant change. In conclusion, laying quails had the breed-specific characteristics in response to dietary CLA. And high level of CLA supplement ($> 1.5\%$) has adverse effects on egg quality and DHA content of yolk.

Keywords: quail; conjugated linoleic acid; egg quality; cholesterol; n-3 PUFA

S9- 0031 Proteomic comparison by iTRAQ combined with mass spectrometry of egg white proteins in laying hens (*Gallus gallus*) fed with soybean meal and cottonseed meal

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Replacement of Soybean meal (SBM) with cottonseed meal (CSM) has been one research field in animal feed industry due to supply shortage and increasing price of SBM. The depressed albumen quality of eggs induced by CSM supplementation were reported by many previous studies. However, the molecular mechanism of this depression is unclear yet. A total of 216 40-wk-old Hy-line W36 laying hens were given 3 dietary treatments with six replicates of 12 birds each. The control group was fed SBM basal diet, and the other two experimental diets were with 50% (CSM50) and 100% (CSM100) of the dietary protein content provided by SBM replaced by CSM. The feeding trial lasted for 12 weeks. iTRAQ proteomic tools was implemented for study of egg white proteins from hens fed CSM and SBM. We had the following findings. 1) Feed intake, egg weight, albumen weight, albumen height, and Haugh unit were depressed in CSM100 group compared with SBM group. 2) A total of 15 egg white proteins were found with significant decrease in intensity in the CSM100 group including ovalbumin, ovotransferrin, ovomucin, lysozyme, ovomucin, clusterin etc. These decreased proteins accounts for 75% egg white proteins and are involved with various biological functions, which play an important role on protective mechanisms of egg white. This may relate to the reduction of albumen quality in the CSM100 group. 3) A reduced level of plasma progesterone caused reduced growth of the tubular gland and epithelial cells in the magnum, further decreasing egg white protein synthesis in the magnum. 4) These findings, at the molecular level, showed that SBM should not be totally replaced with CSM in a hen diet.

Keywords: albumen quality, cottonseed meal, egg white proteins, iTRAQ, hens

S9- 0032 Understanding egg cuticle formation: a route to reduce egg contamination

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To understand the factors affecting cuticle formation to help improve its quality. The cuticle of the egg is a glycosylated protein layer which covers the outside of the eggshell. It forms a defence to the transmission of micro-organisms and its variability determines protection. About 30% of cuticle variation is genetic but little is known about other sources of variation with confusion regarding the relationship, if any, between the cuticle and pigment. Lohmann hens laying brown eggs were maintained in pens or transferred to cages on 14L: 10D. To accurately determine oviposition time hens were put on a 14L: 14D lighting pattern to synchronise ovulation/oviposition. Pigment and cuticle deposition were quantified by reflectance spectroscopy and staining with Tartrazine/Lissamine green. Experiments were: 1) Pen to cage transfer; 2) Comparison of premature oviposition using Gonadotrophin Releasing Hormone (GnRH) or Arginine Vasotocin (AVT); 3) Time of AVT injection prior to expected oviposition; 4) The effect of the pigment removing coccidiostat Nicarbazin. The mild stress of pen to cage transfer caused a small but significant ($P=0.049$) decline in cuticle but not in pigment. Eggs induced prematurely by GnRH had normal cuticle compared to AVT induced eggs laid at the same time which had virtually no cuticle ($P<0.001$). Eggs induced at 1 hr prior to the expected oviposition had little or reduced cuticle but pigment was less affected. Nicarbazin removed pigmentation but cuticle cover was not affected. Conclusion: Mild stress has a limited effect on the cuticle. Induction of premature oviposition suggests that a normal endocrine cascade produced by GnRH is required to produce a fully formed cuticle while the events invoked by AVT, which are essentially in the shell gland, are not sufficient. The cuticle is deposited in the last moments before oviposition and the event can be separated from pigment deposition.

Keywords: cuticle, eggshell, spectroscopy

S9-0033 Effect of glycation on structure and gelling strength of egg white protein

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Introduction: There is a great demand in egg white protein(EWP) of excellent gelling strength with the development of food industry. However, gelling properties of EWP significantly reduced with prolonging storage time during shelf life. Glycation is a useful and promising method for the improvement of gelling properties and storage stability. The gelling properties of glycation egg white protein (GEWP) were related to structure. A analysis in changes of GEWP structure was in favor of understanding the mechanism of improving gelling properties of EWP through glycation. **Methods:** The gelling strength and structure of GEWP were measured using texture analyzer, SDS- PAGE, fourier transform infrared spectroscopy (FTIR), circular dichroism spectra(CD), X-ray diffraction, ultraviolet absorption spectrum(UA). **Results and discussion:** The gelling strength greatly increased to $1240.73 \pm 45.83 \text{ g/cm}^2$ under 1% addition ratio of polysaccharide to EWP, heat treatment at 80°C for 5 days. After storage of 6 months, the gelling strength of GEWP was above 1000 g/cm^2 , suggesting GEWP had excellent storage stability. SDS-PAGE revealed that ovotransferrin and ovalbumin glycated with polysaccharide. FTIR analysis indicated that polysaccharide was linked to protein covalently. The results of CD correspond with that of FTIR, only slightly different in data. In comparison with EWP, the content of α -helical and β -turn of GEWP decreased, but that of β -sheet and random structure increased. Tertiary structure of EWP was also modified by glycation. The decrease of tryptophan fluorescence intensity of GEWP suggested the GEWP converted to unfold conformation. X-ray diffraction showed that GEWP contained several compounds with nature of crystal. UA showed that amino acid was changed by glycation and hydrogen bond in GEWP solution increased.

Keywords: egg white protein, glycation, gelling strength, structure

S9-0034 Influence of dry-heating on gelling and structural properties of ovalbumin

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Introduction: Dry- heating (DH) is one of the most promising approaches for improving the gelation of egg white powder (EWP) that is an important ingredient in food processing. However, the mechanisms responsible for the markedly improvement of gelation of EWP are not fully explained because of complicated interactions among the different proteins. Therefore, monitoring the changes in protein structure of ovalbumin (OVA) upon DH processing, the main constituent of EWP, will be conducive to the understanding of the relationship between the structure and gelling properties of EWP. **Methods:** OVA was heated in the dry state for 0-30 days at 75°C and 30 percent humidity. The gel strength and structural properties of dry- heated OVA were investigated using texture analyzer, scanning electron microscope, laser particle size analyzer, UV spectrophotometry and others. **Results and discussion:** The gel strength greatly increased to the highest point ($1129.83 \pm 86.09 \text{ g/cm}^2$) on the 21th day, and then decreased. Microstructure images and electrophoretic patterns revealed the formation of OVE polymer through hydrophobic and disulfide protein-protein interaction during DH. Average particle size and enthalpy of denaturation reduced with increase of DH time. The slight changes in ultraviolet spectrum indicated mild conformations changes in OVE molecules. The absolute values of zeta potential increased after 21 days. It was assumed that the proper unfolded OVE with smaller particle size distribution produced by DH forms specific soluble aggregates during subsequent heating for gelation, resulting in the formation of an ordered gel matrix. But increase of intermolecular forces led to weak gel.

Keywords: ovalbumin, dry- heating, gelation, structural properties

S9-0035 Comparison of methods to evaluate egg freshness: HU vs YI and digital vs manual

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In Japan eating raw egg is not unusual. Therefore a simple, fast and accurate method to evaluate egg freshness is needed. Egg freshness can be measured using the Yolk Index (YI), or more commonly using the Haugh Unit (HU), which has been accepted internationally as an index of egg freshness. The objective of this study was to compare two methods for evaluating egg freshness: HU vs YI and manual vs digital measurement in terms of these accuracy and simplicity. An egg quality gauge and a vernier caliper were used in the manual measurement. The Digital Egg Tester (DET6000) (Nabel Co., Ltd, Japan) was used in the digital measurement. [Methods] Four hundred and two eggs in various freshness states were tested. Eggs were weighed and cracked. In the digital measurement condition, egg freshness was measured using the DET6000 and expressed as HU and YI. In the manual measurement condition, the thick egg white height was measured using the egg quality gauge and expressed as HU. Then the height and diameter of egg yolk were measured using the egg quality gauge and the vernier caliper and expressed as YI. The correlation between manual and digital data (HU, YI), and the correlation between HU and YI were analyzed using least-square method. The correlations between digital and manual measurements were found to be $R^2=0.72$ in HU and $R^2=0.90$ in YI, respectively. YI had a stronger positive correlation than HU. Based on the analysis of coefficient of variations, the dispersion of YI data was significantly smaller ($P<0.001$) than that of HU by Student's t-test. In conclusion, YI is a better accurate index to determine egg freshness than HU. Although HU has been generally accepted as an index to evaluate the freshness of eggs, our results suggest that YI should be used as an authentic standard to evaluate egg freshness with much reliability.

Keywords: Haugh Unit, yolk index, egg freshness, digital egg tester

S9-0036 Determination of 5 sulfonamides veterinary drugs residues in eggs

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Objective To establish a method for simultaneous determination of sulfamethazine (SM2), sulfamonomethoxine (SMM), sulfamethoxazole (SMZ), sulfadimethoxine (SDM), sulfaquinoxaline (SQ) residues in eggs by RP-HPLC coupled with solid-phase extraction (SPE). **Materials and methods** The residues in the samples were extracted with ultrapure water and acetonitrile, and cleaned by solid-phase extraction (MCX 60mg 3cc), and then the eluant was evaporated to dryness under a stream of nitrogen, and redissolved by the initial mobile phase. The redissolved liquid was degassed by n-hexane, and separated by RP-HPLC on a XBridge-C18 ($4.6 \times 250\text{mm}$, $5\mu\text{m}$) column using acetonitrile-methyl alcohol-ultrapure water-acetic acid ($2+2+9+0.2$, V/V) as mobile phase at a flow rate of 1.0mL/min , and detected with a ultraviolet detector. The UV detection wavelength was set at 270nm and the column temperature was 30°C . **Results** Under the optimal experimental condition, the target compounds had good chromatographic peak shapes and separation degrees. The calibration curves were linear in the range of $20.0\text{ng/mL} \sim 1000.0\text{ ng/mL}$ for determination of 5 sulfonamides residues in eggs and the linear correlation coefficients were more than 0.999. The method detection limits ($S/N=3$) for analytes ranged from $7.5\mu\text{g/kg}$ to $20.0\mu\text{g/kg}$, the limits of quantitation ($S/N=10$) were in the range of $25.0\mu\text{g/kg} \sim 65.0\mu\text{g/kg}$. The relative standard deviations (RSDs) were not more than 3.7% and the recoveries for the spiked samples varied from 75.9% to 96.5%. **Discussion and conclusion** The results indicated that this method was easy to operation and efficient technique without complex sample pretreatment steps. Thus it could be applied to the simultaneous determination of 5 sulfonamides veterinary drugs in egg samples and meet the requirements for the quality monitoring of eggs and daily mass rapid analysis.

Keywords: sulfonamides veterinary drugs; eggs; solid-phase extraction; high-performance liquid chromatography

S9-0037 Polyphasic characterization of Salmonella Enteritidis isolates on persistently contaminated layer farms during the implementation of a national control program with obligatory vaccination: a longitudinal study

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Since 2007, a national Salmonella control program including obligatory vaccination has been ongoing in Belgium. In this context, the aim of the present study was to investigate the diversity of Salmonella Enteritidis (SE) isolates on five persistently contaminated Belgian layer farms and to examine the potential sources and transmission routes of SE contamination on the farms during successive laying rounds. A collection of 346 SE isolates originating from the sampled farms were characterized using a combination of multilocus variable number of tandem repeat analysis (MLVA) and phage typing (PT). On each farm, one or two dominant MLVA-PT types were found during successive laying cycles. The dominant MLVA type was different for each of the individual farms, but some farms shared the same dominant phage type. Isolates recovered from hens' feces and ceca, egg contents, eggshells, vermin (mice, rats, red mites and flies) and pets (dog and cat feces) had the same MLVA-PT type also found in the inside henhouse environment of the respective layer farm. Persistent types were identified in the layer farm inside environment (henhouse and egg collecting area). Furthermore, this study demonstrated cross-contamination of SE between henhouses and between the henhouse and the egg collecting area. Additional isolates with a different MLVA-PT type were also recovered, mainly from the egg collecting area. A potential risk for cross-contamination of SE between the individual layer farms and their egg trader was identified.

Keywords: Salmonella Enteritidis, layer farms, polyphasic characterization, longitudinal study

S9- 0039 Effect of pasteurized and unpasteurized eggs on quality characteristics of pancakes

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Egg has an important role in a well-balanced diet due to its essential nutrients such as proteins, vitamins, and minerals. It is also a desirable ingredient which is widely used in cakes, pancakes, mayonnaise, and pastries. In this study, the effects of usage of pasteurized liquid eggs having different Brix contents (21, 23, 25 Brix) and unpasteurized standard and organic whole eggs in pancake quality were investigated. Brix, pH, color, foaming capacity, and foaming stability of the eggs were determined. All the pancakes were prepared using the same recipe and cooking conditions. Color and pH of pancake batters were measured. Color (L*, a* and b*), texture (hardness, cohesiveness, springiness, and chewiness), and sensory properties (appearance, color, odor, texture, taste, and overall acceptability) of pancakes were analyzed using a colorimeter, texture analyzer and taste panels, respectively. Standard whole and organic eggs Brix were 26.4 and 27.2, respectively. The pH values of eggs varied from 7.73 to 7.86. Foaming capacities and foaming stabilities were ranged from 570 to 690 ml and from 28.5 to 41.4%, respectively. The highest L* value was observed in pancake prepared by 21 Brix pasteurized eggs due to high amount of egg white while pancakes prepared by organic eggs had the highest b* value due to egg yolk color. Pancake prepared by 23 Brix pasteurized eggs had higher values for textural properties compared to others. Based on sensory scores, the most preferred pancake was the one made from 21 Brix pasteurized eggs with high scores in color and odor and this could be due to the presence of less egg yolk and more egg white in 21 Brix pasteurized egg composition. Pancake prepared with organic unpasteurized eggs was the least preferred sample with low scores in color and taste. As a conclusion, low Brix (21 and 23) pasteurized liquid eggs could be recommended in preparation of pancakes due to their effects on sensorial and textural qualities of pancakes.

Keywords: pasteurized eggs, unpasteurized eggs, pancakes, sensory analysis

S9- 0040 Research in sedimentary and change rule of n - 3 PUFA in poultry products

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In order to explore the n-3 PUFA sedimentary rule in laying hens and chicken, and obtain the influence of n-3 content in eggs with different storage methods, We carry out the n-3 eggs production feeding experiment, on the basis of early omega- 3 type wheat diet research. This test choose JingBai 939 as the test animal, it is divided into 10 experimental groups, 1 control group, each group includes 96 chickens, a total of 1056 laying hens, the test period is 6 weeks. The results show that the precipitation of fat in the eggs with the extension of test time change was not significant ($P > 0.05$), n - 3 (ALA, DHA and EPA) content changing with feeding weeks in eggs is on the rise, compare to 1 week of treatment, the precipitation of fat in egg was significantly increased after 2-6 weeks ($P < 0.05$). Different storage methods had no significant effect ($P > 0.05$) on total fatty acid content, but for n - 3 PUFA, the precipitation of fat room in temperature storage for 2-5 weeks is significantly lower than in cold storage and the control group ($P < 0.05$). Under the condition of 1 and 6 weeks of storage, the n - 3 PUFA level had no significant difference ($P > 0.05$) between different ways of storage. The abdominal fat and abdominal fat percentage of the test chicken are rising, it had significant difference ($P < 0.05$) after 2 weeks treatment; the n - 3 PUFA level in leg muscle was significantly increased ($P < 0.05$) In feeding 3 weeks, but for breast muscle, the critical point are 4 weeks, and n - 3 PUFA content is continuous increased in both leg muscle and breast muscle as the extension of feeding date.

Keywords: n-3 PUFA, sedimentary, change rule

S9-0041 Functional studies with recombinant forms of the eggshell matrix protein Ovocleidin-17

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Chicken ovocleidin-17 (OC17) is a strong candidate to control and regulate the deposition of calcium carbonate in the calcified eggshell. Computer simulations based on the crystal structure of OC17 protein, proteomics analysis and mRNA expression results suggest that it may act as a catalyst in the transformation of amorphous calcium carbonate (ACC) to crystalline calcite. Aim of this study was to test the role of wild-type OC-17 and mutated OC-17 protein in the transformation of ACC and the potential functional sites. Therefore, we have expressed and purified recombinant wild type (O) and mutated OC17 (S) in order to perform structure / function studies to elucidate its interaction with calcium carbonate mineral. The 293T cell line was used to express full-length OC17 cDNA for the wild type and OC17 sequence mutated to delete a key amino acid (Ser-61). Calcium carbonate precipitation was performed by vapour diffusion from 0.2M CaCl_2 , in the presence of purified recombinant proteins (10 - 100 $\mu\text{g/ml}$). The harvested crystals were washed and examined by SEM, FTIR and XRD. Results: SEM - In control experiments, calcite and vaterite are formed; the number of crystals decreases with increasing protein concentration. There is oriented nucleation of calcite crystals on (001). ACC is likely present but cannot be distinguished. FTIR: Initially, calcium carbonate is ACC and later on there is a mixture of calcite and vaterite, with smaller proportions of ACC. In O protein experiments only calcite is observed. In S-mutant experiments there is also a minor amount of vaterite at longer times (45-60 min). XRD: difficult to interpret due to preferential orientation of crystals which would not reflect. In conclusion, OC-17 play roles in the transforming ACC to crystalline calcite. This strategy will allow key residues and functional domains of OC17 to be defined.

Keywords: calcite, biomineralization, recombinant protein

S9- 0042 Development of a method for prediction of eggshell damage in order to improve egg handling and packing processes

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Increasing the maximum age of laying hens in order to produce more top quality eggs per hen housed, will increase the risk of egg shell damage due to a loss of shell strength. This raises the question of how egg shell damage occurrence may be reduced during collection, packing and transport. We performed a model based study focused on hairline cracks in eggs of 88 week old hens, and simulated side collisions on 1235 eggs using a specially designed pendulum. The kinetic energy at the moment of impact was related to the accelerations measured inside the transport chain by an electronic egg. Further, several egg mechanical properties were measured. Our model correctly predicted that impacts of 3.4 mJ, typical for automated egg handling processes in the Netherlands, result in a crack percentage of 7.7%. Our model predicts that a moderate decrease of 30% in impact energy will result in a drastic reduction of crack occurrence, from 7.7% down to 0.3-1%, whereas an increase of 30% will increase crack occurrence to 42-55%. These predictions show a relatively high sensitivity of crack occurrence towards collision severity. The combined model predictions and correlations imply that, under current operational circumstances, collisions play a far more important role in hairline fractures than the measured egg properties. This suggests that to reduce hairline cracks, a reduction of collision severity is of first priority when increasing the age of laying hens.

Keywords: egg quality, electronic egg, eggshell damage

S9-0043 The Effect of different immune adjuvants on the immunogenicity of laying hens

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Specific egg yolk immunoglobulin (IgY) has gradually become a promising alternative to antibiotics in the prevention and control of bacterial disease as it possesses many advantages, including safe, high efficiency and easy preparation. However, low antibody titer and production rate is one of the problems to be solved in the study of IgY. For screening immunologic adjuvants of high efficiency, security, low cost, easy injection, low side effect suitable for egg-laying hen to solve it, this experiment will take ETEC K88 standard strain as model antigen, respectively blended with different CpG-ODN (F1-F5) sequences which are synthesis of artificial design, Astragalus polysaccharides, Chitosa and Freund's adjuvant to make inactivated vaccines, immuned brown crust egg-laying hen. 40 120-day-old chickens were randomly divided into 10 groups. All the chickens were immunized three times. Blood samples were collected to measure IL-2 secretion and IFN- γ secretion by ELISA test respectively. Results showed that CpG-ODN could induced IL-2 and IFN- γ expression obviously, especially CpG-ODN F2. The highest expression yield were 38ng/L and 54 ng/L, respectively. After the third immunization, the egg productions of Group chitosa, FA (Freund's adjuvant), and Propolis decreased more than the other groups. Enzyme-linked immunosorbent assay (ELISA) results showed that the highest IgY titer was up to 360000 and the IgY titer remained stable till 9 week in F2 groups. Thus we came to a conclusion that Group CpG-ODN F2 and Astragalus polysaccharides were better to be developed as potential adjuvants for egg yolk antibodies production.

Keywords: CpG-ODN; IL-2; IFN- γ ; egg yolk immunoglobulin (IgY)

S9- 0044 Influence of lycopene on pigments concentration, malondialdehydes and fatty acid profile in the egg yolk, using sunflower, rapeseed and linseed oils in laying hens nutrition

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This study aimed to evaluate the effect of using lycopene and oils in diets for laying hens on the egg quality as pigments concentration, malondialdehyde (MDA) and fatty acids, on laying hens. Lohman Brown laying hens which were 30 weeks old were assigned to 6 treatment groups and fed with the experimental diets for 8 weeks. The compound feed were supplemented with different oils (sunflower, rapeseed and linseed) and in experimental groups also were insert 25g/kg lycopene and keeping in the same conditions. MDA concentration and pigments in the egg yolk were determined by high performance liquid chromatography as described by Mendes (2009), with a high pressure gradient HPLC system (Varian ProStar). Fatty acids concentration were determined with gas chromatograph Shimadzu GC -2010 Plus. Statistical Analysis. The results of the experiment were analysed using the 1-way ANOVA test, and significant differences between groups were determined by Duncan's multiple range test. Statistica 8.0. for Windows™ software was used. Differences were considered significant at $P < 0.05$. Statistical significant results of fresh eggs were: MDA concentration, in all experimental groups it decreased from 39 to 57% ($P < 0.05$), storage at 28 days this parameter lycopene did not have effect. Linseed oil with lycopene statistically significant increase pigment concentration and total of monounsaturated fatty acids concentration (MUFA), it increased 1.97 percent ($P < 0.05$) compared with I control group, PUFA in I experimental group increased 2.37% ($P < 0.05$), but in II experimental group it decreased - 3.49% ($P < 0.05$) compared with control groups. Omega 3 increased in all experimental groups from 1.75 to 3.75 ($P < 0.05$), compared with control groups. The results of this study clearly demonstrate that supplementation of lycopene and different oils had effect on MDA and pigment concentration, MUFA, PUFA and omega 3 concentration on egg yolk.

Keywords: eggs, fatty acids, pigments, MDA

S9- 0045 Effect of washing, storage condition and storage duration on quality of shell eggs

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The main quality feature of chicken eggs is freshness which can be assessed by determining air chamber height (non-destructive) and by measuring albumen height (destructive) and calculating Haugh units. In a fresh laid egg air chamber height is low and albumen height is high. During storage air chamber height is continuously increasing and albumen height is decreasing due to the evaporation of water and loss of carbon dioxide. Under normal storage conditions eggs may be stored for 4- 6 weeks. Under low temperature, increased humidity and high carbon dioxide atmosphere the storage duration may be prolonged to several months. In some countries washing of eggs is done to improve shelf-life. By this, the cuticle may be removed from egg shell, egg shell pores are opened and evaporation processes increase. Therefore, washed eggs are normally stored under low temperature conditions. The objective of the study was thus to investigate the effect of hen age, washing, storage temperature and storage duration on the quality of chicken eggs. Eggs of 35 and 65 weeks old LSL hybrids were stored under a temperature of 6, 15 and 22°C for 3, 18 and 28 days. The effect of washing was investigated only in 65 weeks old hens. Egg mass, shell weight, shell thickness, air chamber height, albumen height and Haugh units were determined in 800 eggs, in total. Hen age did not distinctly affect quality features of the eggs. Loss in egg mass increased with storage duration and was highest for 22°C. In the same way, air chamber height increased and albumen height and Haugh units decreased with duration of storage. The biggest effects were observed for storage at 22°C. Washing of eggs resulted in a higher increase in air chamber height at 22°C, independent of storage duration. Similar effects were observed for albumen height and Haugh units. Results indicate that a lower storage temperature assures freshness of eggs for a longer time, especially if eggs have been washed.

Keywords: egg, freshness, storage condition, storage duration, washing

S9-0046 Effect of bee bread supplementation of diet on laying hen performance, egg quality, sensory traits and fatty acid composition

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The aim of the research was to use a bee bread (BB) as a natural feed supplement for laying hens in order to improve performance, egg quality, sensory traits and fatty acid composition. A total of 36 laying hens (Hy-Line Brown) was randomly distributed into 2 dietary treatments with 6 replicates, each comprising 3 hens. Freeze-dried bee bread was crushed and used at the level of 0 and 5% in the diet of the control (0% BB) and treatment group (5% BB), respectively. The feeding experiment was conducted for 4 weeks and at the end of it three eggs from each cage were collected to determine the weight of a whole egg, eggshell breaking strength, Haugh units, yolk color and shell thickness. Sensory properties were evaluated according to a 5 grade scale of acceptance. During the analysis of fatty acid composition the content of saturated, monounsaturated and polyunsaturated fatty acids as well as the ratio of n6 and n3 fatty acids in eggs collected from each group were determined. The total polyphenols content and antioxidant activity (DPPH) of bee bread were measured. The results showed that bee bread supplementation of diet at the level of 5% did not affect hens' body weight and feed intake. Also no differences in the mean egg weight, Haugh units and yolk color and shell thickness were noted in BB-supplemented group. Whereas in case of eggshell breaking strength, significant increase was observed for diet containing 5% of BB. Sensory analysis of eggs did not reveal any differences among treatments. The results showed that bee bread supplementation of diet at the level of 5 % increased eggshell breaking strength and did not negatively influence birds' health conditions as well as quality and sensory traits of eggs. This research was financially supported by the framework of the statutory research No B010/003/15.

Keywords: laying hen, bee bread, fatty acid, egg traits

S9-0048 Investigation and study of amino acid content in eggs from different chicken breeds and different feeding models

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Determined different egg yolk weight, egg white weight and 17 kinds of amino acid content from different chicken breeds and different feeding models by using amino acid auto analyzer. 10 eggs laid by 3 kinds of developed breeds and 3 kinds of native breeds were collected for determining, all kinds of developed breeds are cage-reared, 1 kind of native breed is scattered-feed, and other 2 kinds of native breeds are both cage-reared and scattered-feed. It shows that amino acid contents in egg yolk are significantly higher than egg white. The amino acid contents in egg yolk, egg white and whole egg between different breeds are significantly different. The amino acid contents in egg yolk produced by scattered chicken and cages-reared chickens are not significantly different, but amino acid contents in egg white and whole egg produced by scattered chicken are significantly higher than cages-reared chickens ($P < 0.05$). There was significant interaction between breeds and feeding models on amino acid contents in egg yolk and whole egg ($P < 0.05$).

Keywords: egg quality, amino acid, breed, feeding model

S9-0049 Investigation and study of trace element content in eggs from different chicken breeds and different feeding models

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Determined 40 kinds of trace elements from different chicken breeds and different feeding models by using ICP-MS. 5 eggs laid by 3 kinds of developed breeds and 3 kinds of native breeds were collected for determining, all kinds of developed breeds are cage-reared, 1 kind of native breed is scattered-feed, and other 2 kinds of native breeds are both cage-reared and scattered-feed. Trace elements were divided into essential trace elements, which including Fe, Co, Cu, Zn, Se, Mo, I, Mn, Si, B; toxic trace elements, which including Cr, As, Cd, Hg, Pb; and other trace elements. The result shows that contents of Fe, Cu, Zn, Se, Mo, I, Mn, Si were concentrated in egg yolk, contents of Co and B were concentrated in egg white. The contents of Pb in all 3 developed breeds samples were higher than 200 μ g/kg, which exceeded the limits of national standard. The contents of Cd and Hg were not detected in all samples. There was significant interaction between breeds and feeding models on some trace elements contents in egg yolk and whole egg ($P < 0.05$).

Keywords: egg quality, trace element, breed, feeding model

S9-0050 Inhibitory effects of green tea on the formation of heterocyclic aromatic amines in stewing eggs

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Heterocyclic aromatic amines (HAAs), a kind of carcinogenic and/or mutagenic organic compound, are formed during cooking of eggs. The object of this study was to measure 9 HAAs in stewing eggs with or without tea leaves. Solid phase extraction method was applied for extraction of the HAAs. Chromatographic analysis of HAAs were performed by using an Agilent 1100 liquid chromatograph 1100 (Agilent, California, USA) equipped with a UV detector, fluorescence detector and an automatic liquid sampler. Lipid oxidation in yolk was assayed with TBARS (Thiobarbituric Acid Reactive Substance Assay). Maillard reaction was assayed with absorbance at 420nm. One way ANOVA of GLM process and Pearson correlation was analyzed using SAS 9.4 edition. The results indicated that the amount of HAAs were positively correlated with the boiling time ($P < 0.001$) irrespective of whether tea leaves were added or not. Six HAAs (PhIP, MeIQ, MeIQx, Trp-p-1, Trp-p-2 and AaC) with a total of 8.23 ng/g were detected in eggs for 30 min boiling in water. Seven HAAs (PhIP, MeIQ, MeIQx, Trp-p-1, Trp-p-2, Harman and AaC) with a total of 20.65 ng/g were detected for 30 min boiling in stewing soup (3% salt, 3% sugar, 2% soybean sauce, 1.5% spice (anise:cinnamon:ginger=1:1:1)). However, when 0.75% green tea leaves were added in stewing soup, only 4 HAAs (MeIQ, MeIQx, Trp-p-2, and AaC) with a total of 4.78 ng/g were detected, exhibited significantly lower as compared to eggs in boiled water and stewing soup for 30 min ($P < 0.001$). Consistently with the decrease of HAAs in eggs boiled with tea, the TBA from yolk was also reduced from 6.45 (eggs boiled in water) to 2.30 mg/kg. Further measurement of ABS420, an index of maillard reaction, detected a significant decline ($P < 0.01$) which reflected the inhibitory effect on the formation of HAAs in eggs might correlated with the inhibition on maillard reaction of green tea.

Keywords: stewing eggs, heterocyclic aromatic amines (HAAs), green tea

S9-0051 Production performance of commercial layer influenced by body weight losses during molting

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Molting is a natural phenomenon in birds to rejuvenate their potential and body reserves. Commercially molting is being practiced to extend the production cycle as well as reduces the cost of rearing the replacement pullet and increase egg size. This study evaluated the effect of different body weight losses during molting on production performance of commercial layer (LOHMANN LSL-LITE). To this end, a total of 72 birds were arranged according to randomized complete block design and divided into 3 treatment groups having 3 replicates of 8 birds each. Treatment consisted of three body weight losses during molting i.e., 20, 25 and 30%. The bird loss 20% body weight during molting showed better production % and improve FCR / kg egg mass and / dozen eggs. However, large egg size and better egg mass was observed in bird loss their 30 % body weight during molting. There was no influence of body weight loss during molting on feed intake throughout the experimental period. It was concluded that 20 % weight loss during molting had better and positive effect on the performance of commercial layers.

Keywords: commercial layer, body weight losses, molting, production performance

S9-0052 Assessing body conformation of hens at the end of laying period using computed tomography scanning

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The aim of the present study was to evaluate body composition of laying hens, in relation to flock uniformity, at the end of the laying period for hens on a commercial farm and from a laboratory experiment with three different body weight groups at point of lay (light, medium and heavy). A total of twelve birds was randomly selected for the on-farm study at a cage housing system at 80 weeks of age, and eighteen birds from the laboratory experiment with 6 birds per body weight group. Body weight (BW) was measured prior to scanning using an electronic weighing scale (VEIT electronics Poultry scale BAT 1), and the whole body of each hen was scanned using a GE HiSpeed QXi 4 slice CT scanner. The acquisition parameters of the CT scanner were performed with helical scanning 120 kV, 140 mA, 5 mm thickness, 5 mm spacing and 1 second scanning time. The abdominal fat depots were also weighed. For the on farm birds, BW at 80 weeks of age ranged from 1.9 kg to 2.5 kg. There was a significant positive correlation between BW and abdominal fat pad ($R^2 = 0.8076$), a significant negative correlation between BW and lean composition ($R^2 = 0.6279$) and no significant correlation between BW and percentage bone in the body. For the laboratory experiment, BW ranged from 1.9 kg to 2.7 kg. There was no significant difference among the BW groups for body weight, abdominal fat pad, and variables measured by CT. However, there was a significant positive correlation between body weight and abdominal fat pad both measured and predicted by CT ($R^2 = 0.5125$ and $R^2 = 0.5875$, respectively). There was also a significant negative correlation between BW and lean composition ($R^2 = 0.3874$), and BW and bone percentage ($R^2 = 0.2678$) as measured by CT. Body fat content increased linearly with body weight, while the composition of lean and bone decreased as body weight increased. CT is an accurate method for measuring body conformation of laying hens.

Keywords: laying hens, computed tomography, body conformation

S9- 0053 The effect of white or brown layers reared and tested in the same conditions for comparison of egg quality parameters

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The quality of the eggs is influenced by many factors. These factors can be classified into internal (e.g. genotype, age and weight of hens, oviposition time) and external (e.g. housing system, nutrition and temperature). Genotype often act in interaction with other factors. The objective of the present work was to compare the egg quality of brown-egg laying type hens Dominant brown D102 and Dominant leghorn D229, which have white egg. For analysis 300 eggs were collected from each genotype (35 weeks of age of hens - peak of laying period). The eggs were weighed using an electronic scale with 0.01 g sensitivity. Eggshell strength was determined by the analyser Instron Model 3342 manufactured by Instron United States. The proportions of yolk, albumen and eggshell were calculated in relation to egg weight and expressed as percentages. The egg shape index and Haugh units score were computed as well. The statistical analysis was processed by the computer application SAS 9.3 with GLM procedure for all parameters. The effect of genotype was included in the model for egg quality characteristics. Significantly higher egg weight was found in Dominant brown D 102 (64.57 g), compared with Dominant D 229 (59.98 g). Significantly higher egg shape index, percentage of albumen and index of yolk was observed in brown-egg laying hens Dominant brown D 102 (76.99 vs. 75.28 %, 63.68 vs. 61.84 %, 46.59 vs. 43.78 %, resp.). On the other hand, white- egg laying hens Dominant leghorn D229 were detected conclusively higher percentage of yolk and eggshell, eggshell strength and its thickness, compared to Dominant D 229 hens (28.36 vs. 27.27 %, 9.80 vs. 9.05 %, 41.85 vs 36.15 g/cm², 0.35 vs. 0.33 mm, resp.). Albumen index and Haugh units score were insignificantly higher in white-egg laying hens Dominant leghorn D229 (6.44 vs. 6.38 %, 71.72 vs. 69.88, resp.). This research was funded by an "S" grant of the MEYS of the CR.

Keywords: layers, dominant, quality, yolk, albumen, eggshell

S9- 0054 The improvement of egg-shell ultrastructure by dietary supplementation of organic manganese in laying hens

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The effect of dietary organic manganese (Mn) supplementation on eggshell ultrastructure in layers were explored. We first examined the effect of dietary supplementation with different levels of organic Mn on eggshell mechanical properties in trial 1. Six hundred 46-week-old Jinghong layers were fed a control diet containing 32.70 mg/kg of Mn for two weeks and then randomly allocated to five groups and fed a control diet or the control diet supplemented with 40, 80, 120, or 160 mg organic Mn/kg of feed. Dietary Mn addition quadratically improved eggshell breaking strength and thickness, and the optimal supplemental level of organic Mn was 80 mg/kg. In trial 2, we further investigated the effect of dietary 80 mg/kg Mn addition on eggshell quality and ultrastructure under different formation processes. A total of 192 54-week-old Jinghong layers were fed a basal diet with Mn level at 29.70 mg/kg for two weeks and then randomly divided into 2 groups. Layers were put into individual cages to record oviposition time each day by automatic monitored control system. At the end of the trial, four birds from each replicate were sacrificed 5, 9.5, 18.5, or 22.5 hours after last oviposition to obtain eggs, respectively. The eggshell breaking strength, thickness, shell ratio and fracture toughness were significantly improved in Mn addition treatment. The width of mamillary cones and mamillary layers' thickness were decreased and the effective thickness was increased by dietary Mn addition. At 18.5 hours after last oviposition, the eggshell breaking strength was significantly increased, and the average size of calcite crystals were decreased in Mn addition treatment. Overall, our data suggest dietary organic Mn supplementation can improve eggshell mechanical properties by modulating eggshell ultrastructure, especially at the rapid growth of the polycrystalline calcite in the eggshell formation.

Keywords: organic manganese, eggshell ultrastructure, laying hen

S9-0055 Change in calcium carbonate mineralogy and in organic matrix proteins quantified by proteomic analysis during the early stages of egg shell mineralization in hens

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Eggshell is a biomineral made of 95% calcite modulated precisely during the phases of initiation, rapid growth and termination of its formation by the proteins of the organic matrix (3.5%). It is a highly ordered structure, with unique mechanical properties, made of large columnar calcite units arising from specific nucleation sites on the eggshell membrane. We explored the profile of proteins and shell mineralogy and crystallinity during the early stages of shell formation (diffuse scattering of X-rays, infrared spectroscopy and HRTEM) and revealed that eggshell mineral originates from the accumulation of flat disk-shaped amorphous calcium carbonate (ACC) particles on specific organic sites on eggshell membrane. These sites are rich in proteins and proteoglycans promoting the nucleation and stabilization of ACC with calcitic short-range order predetermining the calcite composition of the eggshell. The transient ACC forms aggregation of nanoparticles which crystallize into calcite. The abundance of 300 shell matrix proteins was quantified at five steps of calcification. 24 matrix proteins were predominant at a particular stage of shell calcification allowing hypothesis on their functions: OC-17 and ovalbumin were overabundant in the shell at the early stages and are suspected to stabilize ACC; ED-IL3, ALB, MFG8, HPX and NUCB2 are also promising candidates being calcium binding proteins. LOXL2 might be involved in membranes formation. OC-116, HAPLN3, SDCBP, TSKU and GPC4 are proteoglycans known to influence the CaCO₃ biomineralisation. Lysozyme and ovotransferrin were highly present at all stages and might interact with calcium as shown in vitro. OOCX32, showing polymorphism in association with shell quality was overabundant when large columnar calcite crystals develop. Chaperone proteins and protease inhibitors were also revealed. These mechanisms and the interactions of proteins with CaCO₃ might explain the rapidity of shell crystal growth and its properties.

Keywords: egg shell, nucleation, amorphous CaCO₃, matrix proteins

S10-0001 Prevalence and antibiotic resistance of *Campylobacter* spp in broiler flocks in Greece

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Campylobacter is well recognized as the leading cause of foodborne gastroenteritis in humans. In 2008, a European Union-wide baseline survey on *Campylobacter* and *Salmonella* in broiler batches and carcasses was carried out followed by the antimicrobial resistance data on zoonotic and indicator bacteria in 2010. In both studies, Greece did not participate. Therefore, there has been a dearth of information on prevalence and antibiotic resistance of *Campylobacter* in broiler flocks. The aim of this epidemiological study was to estimate the prevalence of *Campylobacter* in broiler farms and to evaluate the antibiotic resistance of *Campylobacter* spp isolates. 10 caeca and 5 neck skin samples from randomly selected carcasses were collected from 142 batches of broiler chickens slaughtered in 3 Greek slaughterhouses over a 12-month period. *Campylobacter* spp were isolated from 73.9% of caeca and from 70.6% of carcasses. By using PCR, 2 species of *Campylobacter* were identified, *Campylobacter jejuni* and *Campylobacter coli*, which were isolated in 35.2% and 64.8% of positive caeca samples, respectively. *C. coli* was also the most frequently isolated on neck skin samples with 57.4%, whereas *C. jejuni* was present in 42.6% of positive carcasses. Positive isolates from caeca and neck skin samples were also analysed for antibiotic resistance with Mueller-Hinton Agar Disk Diffusion Test. In *Campylobacter* isolates from caeca samples, resistance to fluoroquinolones (86.6%) and tetracyclines (80%) was high, while resistance to erythromycin (7.7%) was recorded at low levels. Isolates from neck skin samples also showed a high level of resistance in ciprofloxacin (92.2%) and nalidixic acid (91.2%) and a moderate (59.8%) in tetracyclines, whereas much lower levels were observed for erythromycin (9.8%). This study underlines the presence on *Campylobacter* spp in broiler carcasses and the importance of *Campylobacter* spp reduction on final products due to increased resistance to common antibiotics.

Keywords: *Campylobacter*, prevalence, resistance, Broilers, Greece

S10-0002 Effect of bio-security management intervention on meat quality of different broiler farming in Bangladesh

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The study was undertaken to determine the quality of meat through proximate analysis of broiler farming carried out with and without bio-security management intervention in Rajshahi, Pabna and Kishorgonj districts of Bangladesh. A total of 36 broiler meat samples were collected from broiler farmers with considering bio & non bio-secured managed farms during summer and winter between June, 13 to December' 14 and having 18 meat samples in each season. Categories of farm (bio-secured & non bio-secured) were identified by using measures of bio-security standard which were based on marks. As per bio-security standard of broiler farm, the farms those got above 60 marks treated as bio-secured farms and below 60 marks treated as non-bio-secured farms (out of 100 marks). Data were analyzed using SPSS 11.5 (2003) program and comparisons of results were made between farms with & without bio-secured intervention. No significant differences were found in moisture, crude protein, ash, crude fiber and ether extracts in both seasons among the broiler farm locations. But, between of the two management intervention during summer and winter seasons had a significant effect on proximate parameters. CP, moisture, ash, EE and CF were found higher in bio-secured managed farm in comparison with non bio-secured farm in both seasons. Average proximate parameters of broiler meat samples in both seasons showed the following composition: moisture 76.09%, CP 20.07%, ash 1.07%, CF 0.033% and EE 1.05% respectively considering as fresh basis. Overall relatively better meat quality was found in bio-secured managed farms than those of non bio-secured managed farms. Moreover, between the two seasons, in terms of meat quality, CP, ash and EE were found higher in winter in comparison with summer. It was concluded that satisfactory improve meat quality is achievable from small and medium scale broiler farming at rural households of the farmers if bio-security management intervention is practiced.

Keywords: bio- security, non bio- security, meat quality, winter, summer

S10- 0004 Comparison of nutrient composition and sensory quality of meat of Indian desi-chicken and commercial broilers

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An experiment was conducted to compare the nutritional and sensory quality of Indian desi-chicken and commercial broilers. A total of 20 commercial broilers and 20 Indian desi-chicken were obtained from an organized poultry farm and slaughtered by humane method. Proximate composition by standard protocol and fatty acid composition meat samples were analyzed by gas chromatography using a fused silica capillary column at Animal Feed Analytical and Quality Assurance Laboratory at Namakkal, India and statistical analysis was done by using t-test. The sensory quality was assessed taste panel and the results were recorded on a nine point hedonic scale and statistical analysis was done by using Kruskal - Wallis K - sample non - parametric test. Indian desi-chicken showed significantly ($P<0.01$) higher crude fiber values where as commercial broiler meat samples showed significantly ($P<0.01$) higher fat content than Indian desi-chicken. Thigh muscles of Indian desi-chicken showed higher ash content ($P<0.05$). Calcium content of breast and thigh muscles of Indian desi-chicken showed significantly ($P<0.01$) higher values than commercial broilers. The saturated fatty acids contents were higher in Indian desi-chicken, whereas total omega-3 fatty acids contents were significantly ($P\leq 0.05$) higher in commercial broilers. The samples received from Indian desi-chicken had significantly ($P<0.01$) lower values of sensory scores such as juiciness, tenderness and overall acceptability, whereas meat samples of commercial broilers showed significantly ($P\leq 0.01$) higher overall acceptability sensory scores. It is concluded that Indian desi-chicken had higher values of crude fiber, calcium and saturated fatty acids where as commercial broilers had higher values of fat, polyunsaturated fatty acids especially total omega-3 fatty acids and overall acceptability of sensory score.

Keywords: nutrients, omega-3- fatty acids, sensory quality of Indian desi- chicken meat

S10– 0005 Rates of and reasons for condemnation of Muscovy duck (*Cairina moschata*) carcasses

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The European hygiene regulation requires the condemnation of any unsafe food. However, few data identified and quantified the rates of and the reasons for condemnation, though establishing a link between lesions and sanitary and zootechnical issues in farm is of major importance. We present an overview of rates of and reasons for condemnation of Muscovy ducks in France. The experiment was carried out in 2 voluntary slaughterhouses slaughtering ducks, their size being representative of French slaughterhouses. During one year (Sept 2012 to Sept 2013) all condemnations were recorded on a standardised form and stored in a database following the national reference system of lesions. Condemnation rates were calculated and factors of variation were investigated through an ANOVA. Total condemnation rate is estimated to 1.23 (n=2,381 inspected batches), no partial condemnation being reported. Condemnation rate depends on animal gender (1.59 and 0.68 for males and females respectively, $P < 0.01$) and season (0.87, 1.46, 1.06, 1.52 in autumn, winter, spring and summer respectively, $P < 0.01$). Data do not show any effect of slaughterhouse, production type (standard vs free-ranged) or batch size. The most frequently reported reasons for condemnation are conformation abnormalities (58.6%), cachexia (14.61%) and ascites (14.56%). The type of production is significantly associated with reasons for condemnation ($P < 0.01$): cachexia and ascites are only reported in standard batches (n=2,265 inspected batches) whereas almost half of free-ranged batches are affected by congestion (n=116). No previous data was available in the international literature. Duck condemnation rate is slightly higher than for broilers (1.04) and reasons for condemnation are different (Salines et al., 2015). This stresses the need for leading specific epidemiological studies according to species and developing control operator training in order to improve efficiently the sanitary situation of poultry production.

Keywords: condemnation, Muscovy duck, poultry, slaughterhouse

S10–0006 The effect of supplemental soy and citrus flavonoid on antioxidant and microbial quality of meat in broilers

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Soy and citrus flavonoids are natural antioxidants that are known to exhibit several biological effects including modulation of shelf- life and quality of meat. In present study, dietary effects of soy genistein (SG) and citrus hesperidin (CH) were investigated both individually and in mixed form (SGCH) to modulate microbial and antioxidative quality of meat in broiler chickens. Day-old Arbor Acre broiler chicks (n= 720) were randomly assigned to six replicate pens (20 chicks/ pen) of six treatment groups. Chicks were fed a basal diet without any additive (control), basal diet with 5 mg SG/kg feed (SG5) and basal diet with 20 mg CH/kg (CH20), or basal diet with one ratio four (1:4) of SG and CH with doses of 5 mg/kg (SGCH5), 10 mg/kg (SGCH10) and 20 mg/kg (SGCH20) for six weeks. At the end of experiment, one randomly- selected chick from each pen was euthanized and meat and liver samples were collected. The results showed that both SG and CH treatments significantly reduced ($P < 0.01$) the microbial load of spoilage organisms of breast meat at 0 and 15 d of refrigeration in a dose-dependent manner for all supplemented groups. Fat contents of breast meat were significantly reduced ($P < 0.01$) whereas crude protein was significantly increased ($P < 0.05$) by the dietary treatments with pronounced effects in SGCH20 group. The total antioxidant capacity (TAOC) and superoxide dismutase (SOD) activity was also found to improve ($P < 0.05$) in liver as well as in chicken breast meat particularly in SGCH groups. In conclusion, soy genistein and citrus hesperidin could improve the meat composition, antioxidant and microbial quality of broilers meat in a dose-dependent fashion. In addition, the effects of combined flavonoids supplementation (SGCH) was higher than individual compounds, nevertheless this phenomenon needs to be studied further.

Keywords: broiler, soy genistein, citrus hesperidin, meat quality, antioxidation

S10- 0007 Growth performance, blood characteristics, amino acid pattern and meat quality of broiler chickens fed direct-fed microbial as an alternative to antibiotics

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A total of 800 1-d-old male broiler chicks were randomly distributed into four dietary treatments with four replicate pens per treatment (50 birds/replicate pen). The four dietary treatments fed for 35 d were: a corn-soybean meal basal diet without antibiotic as negative control (NC); NC plus 0.1% virginiamycin as positive control (PC); NC plus 0.1% direct-fed microbials (DFM 1); and NC plus 0.1% mixed direct-fed microbials (DFM 2). No significant differences were found between the treatments for overall growth performance of broiler chickens, but the body weight gain was numerically increased when birds were fed PC and DFM supplemented diets. The levels of glucose and Ca content in blood were not affected by the dietary treatments; however, the total blood cholesterol level was significantly decreased ($P < 0.05$) in PC and DFM supplemented groups compared with the NC group. Dietary DFM significantly increased ($P < 0.05$) the Cystine, Valine, Isoleucine and Proline contents of breast meat of broiler chickens; however, other meat amino acid contents were not affected by the dietary treatments. The Shear force values of breast meat of broiler chickens were not significantly affected by the dietary treatments; however, the cooking loss was significantly decreased ($P < 0.05$) and the water holding capacity was significantly increased in PC and DFM supplemented groups compared with the NC group. The moisture content of breast meat was significantly lower in DFM 2 compared with NC and the lipid content was also significantly lower in PC and DFM supplemented group compared with the NC group. The protein content of meat was not affected by the dietary treatments; however, the ash content of meat was significantly increased in DFM 2 compared with NC. In addition, the DFM supplementation did not affect the tenderness and flavor of breast meat, but the juiciness was significantly higher in DFM 2 than PC. Dietary DFM increases meat quality of chickens.

Keywords: direct fed microbials, blood characteristics, meat quality, meat quality sensory analysis, broiler chicken

S10-0008 The effect of different level of omega-3 and omega-6 fatty acid on performance and meat fatty acids profile of broiler chickens

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An experiment was carried out to evaluate the effects of omega-3 and omega-6 fatty acids and their different ratio on performance and breast and thigh fatty acid composition in male broiler chicken. One hundred and fifty 21-d-old broilers were assigned to 1 of 5 dietary groups. Treatment groups were respectively; basal diet plus 2000 mg/kg omega-6 and basal diet plus 1400, 1800, 2200 and 2800 mg/kg omega-3. Our results indicated that, experimental treatments didn't have significant effect on performance ($P > 0.05$). Experimental treatments had significant effect on chemical composition of breast and thigh ($P < 0.05$). Fatty acid analyzed demonstrated that, all experimental treatments have significant effect on breast and thigh fatty acids profile so that, T1 group had lowest amount of saturated fatty acid (SFA) and T4 group had highest amount of SFA. The omega-3 level increased in breast and thigh meat as omega-3 increased in diet. SFA: PUFA and omega6/omega-3 fatty acid ratio decreased significantly and the lowest amount was observed in T5 group (2800 mg/kg omega-3). The DHA and EPA concentration in breast and thigh increased through omega-3 supplementation. The omega-6 and PUFA concentration in breast meat didn't differ among treatments ($P > 0.05$). Significant difference was not observed in the omega-6 concentration and SFA: PUFA ratio in thigh meat ($P > 0.05$). Omega3 fatty acid efficiency was not affected with dietary treatments. In conclusion, these data demonstrated that, the use of different levels of fish oil (used in this experiment) in diet, didn't have negative effect on performance and carcass characteristics. The fatty acids composition especially omega 3 and PUFA of breast and thigh meat were improved by dietary fish oil.

Keywords: fish oil, Omega 3, Omega 6, meat, enrichment

S10- 0009 Aspects of meat quality and flavour in broilers fed diets containing different oils

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Feeding high levels of unsaturated oils will give unsaturated fat in the meat which is considered a healthy benefit since it is possible to modify the fat in poultry meat by dietary means. However, increasing the degree of unsaturation in poultry meat can give rise to a less stable meat which is prone to oxidize in storage to give unpleasant rancid odours and flavours. The lipid evaluation test is very useful for determining the oxidative status of fat and oils. This study was therefore conducted to evaluate the effect of different dietary oil inclusion in the diet of broiler chicken on lipid oxidation and meat quality. The research focused on the stability of n-3 poly unsaturated fatty acid (PUFAs) enriched meat following cooking and freezing. One hundred and twelve day old broiler chicken (Abor-acre) were randomly allotted to one of six diets of different oils which include T1 (control) no oil, T2 (fish oil) T3 (palm oil), T4 (soybean oil), T5 (palm kernel oil) and T6 (thevetia oil). At the end of eight weeks, four birds were randomly selected from each treatment, slaughtered and dressed for lipid evaluation test and sensory evaluation. Some of the samples were frozen at -4°C for 10 days before cooking. Cholesterol oxidation products (COP), TBARS and peroxide values were measured on raw, frozen and cooked meats. Triangle testing according to British standard was used for the sensory assessment. There was significant difference in peroxide values and TBARS among the treatments ($P < 0.05$) with broiler meat fed Thevetia oil having the highest value of 0.156 and 2.688 u/kg respectively. There was similarity in the total COPs content among the treatments. Cooking generated more oxidative damage than freezing compared to raw sample. Trained sensory assessors were able to detect differences between broiler meats fed different oils with palm oil having the highest overall acceptability. There was detestable effect on the flavor of broiler meat fed Thevetia oil diet.

Keywords: fatty acids, oils, lipid evaluation, sensory, thevetia

S10-0010 Assessment of present bio-security practices in live poultry markets in some selected areas of Bangladesh

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A study was conducted to assess the existing bio-security practices in different live poultry markets in some selected areas of Bangladesh such as Dhaka City Corporation, Sirajgong Sadar and Sahjadpur Upazila by using a pre-tested questionnaire. For his purpose, a total number of one hundred twenty (120) respondents were purposively selected from the above mentioned locations. Forty (40) respondents were selected involve in live bird market and information collected from each area of markets to know the different contributing factors of live bird market bio-security situation through personal interviewing method. Moreover, information's about the present condition of live poultry markets were collected through visual observation. The survey result showed that about fifty percent (50%) respondents used separate dress during working period at their sale centers. Seventy one percent (71%) live birds sellers used different type's drugs to keep their birds sound and alive until sold out all birds. It was also observed that 87 percent respondents did not use commercial disinfectant to clean their dressing materials. This study also noticed that more than 71 percent respondents did not dispose the dead birds scientifically. Further it is noted that the sanitary and hygienic practices of the most live poultry sale centers were poor. This study revealed that there was a high risk of diseases transmission and dissemination both to poultry and human beings due to poor bio-security of live poultry markets in the study areas. It could be concluded that immediate necessary steps need to be undertaken by the government and other regulatory organizations to improve the live bird market situation for ensuring the safe poultry meat in the country.

Keywords: live birds, bio- security, diseases, safety

S10- 0012 Detection of Clostridium perfringens in Brazilian poultry slaughterhouses

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In Brazil, poultry industry is very important been, in 2014, the world's third largest producer, with 12.69 million tons produced. The consumption and production of chicken meat was increased in last decades, this cause a concern with human health due the transmission of harmful pathogens, nevertheless, proper hygiene in the slaughterhouse could inhibit this contamination. The aim of this study was to identify the presence of Clostridium perfringens, in the slaughter line and poultry slaughterhouses equipment's by PCR. 144 poultry intestine samples were collected at slaughter line, processed by opening, removed its contents and scraping of the mucosa, with disposable loop, was done. For the equipment analyses, 251 samples were collected using sterile swabs placed in tubes containing BHI broth, and after plated by pour plate method on RCA agar, incubated at 37°C for 48h under anaerobic conditions. All samples were submitted to DNA extraction by boiling method and subsequently subjected to PCR. C. perfringens was detected by the amplification of specific band for cpa gene. Then, positive samples for cpa gene, were subjected to multiplex PCR amplification for cpa, cpb, etx, iap and cpe genes to detected different types of C. perfringens. From an amount of 395 samples, only two were positive for cpa gene in bacterial identification by PCR, one in slaughter line and one on equipment. The Clostridia positive samples were subjected to multiplex PCR and this method showed that the C. perfringens were type A. This microorganism is part of the natural intestinal poultry microbiota, but becomes a problem if it occurs as carcass contamination by inadequate evisceration. If the hygiene during slaughter and meat processing is performed following the standards established by the Brazilian legislation, there is a greater chance of inhibition of this pathogen and its transmission to humans.

Keywords: aviculture, molecular biology, pathogens

S10-0013 Heat stress relief and meat quality improvement of broiler using post- transportation water- misting sprays with forced ventilation

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Transportation under high temperature was deemed severely stress on broiler which may result in pale, soft and exudative (PSE) meat. There is limited data on the combined effects of water- misting sprays with forced ventilation on broilers after transport during the summer. In this experiment, 45 day old Arbor Acres broilers were transported in a truck for 45 min under the 32°C ambient temperature and were divided into three treatment groups: 1) 45-min transport without rest (T), 2) 45-min transport with 1-h rest (TR), 3) 45-min transport with 15-min water- misting sprays with forced ventilation and 45-min rest (TWFR) in closed shed. Anticoagulant blood and pectoralis major (PM) muscle were collected to detect physiological and meat quality index. PM muscle in the T group reached 53.10 on L* value and would be classified as PSE- like compared with just 47.96 of TWFR group. Drip loss and cook loss of meat from the TWFR group was lower compared with TR group ($P<0.05$). Decreased water mobility of TWFR group was also evidenced by clearly shift towards lower relaxation times of nuclear magnetic resonance (NMR) T21 position, and significantly higher Laman spectrum tyrosine doublet (1850/1830) ratio ($P<0.05$). The results of histology structure and Raman spectrum showed that wider extracellular channels that caused by protein conformation change, may be responsible for higher drip loss of T group. TWFR has lower corticosterone, lactate dehydrogenase, creatine kinase and lactic acid content, while higher Hsp70 and pH among three groups ($P<0.05$). From 0.5h to 1 h postmortem, adenosine 5'- monophosphate (AMP)- activated protein kinase increased higher ($P<0.05$) in the T group, compared with the TWFR group. The results indicated that water-misting sprays with forced ventilation after transport is able to alleviate the stress caused by transport under high temperature conditions during summer and is favorable for the broilers' water holding capacity.

Keywords: broiler, heat stress, meat quality, water-misting sprays with forced ventilation

S10- 0014 Effects of dietary oils on the performance and fatty acid composition of the breast, thigh, and skin tissues of broilers

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Poultry meat is amenable to enrichment with long chain n-3 poly unsaturated fatty acid (PUFA). It is also theoretically possible that LC n-3 PUFA can be synthesized in the body from dietary alpha linolenic acid, the shorter chain n-3 PUFA of plant origin. Fish oils are a rich source of these fatty acids. Given the high world demand for fish oil, there are concerns that the continued and possibly increased use of fish oil in animal diet may not be sustainable and the need for new sources would seem to be vital. Thevetia oil is one of the unconventional sources of oil. The aim of this study was therefore to determine the effect of different dietary oils on performance and fatty acid composition of the breast, thigh and skin tissues of broiler chickens. A total number of one hundred and twenty broilers were randomly allotted into six dietary treatments namely: T1(no oil) control, T2(fish oil), T3(palm oil), T4(soybean oil), T5(palm kernel oil) and T6 (thetvetia oil). After eight weeks of treatment with experimental diets, sample of breast, thigh and skin were taken for analysis. The results revealed that there were significant differences in feed intake, body weight gain and feed conversion ratio. Birds fed fish oil and palm oil consumed more feed compared to those fed thevetia oil ($P < 0.05$). The body weight gain followed the same trend, however birds fed soybean oil had improved feed conversion ratio. Breast and thigh of the birds fed thevetia oil had the highest linoleic, linolenic and alpha-linolenic acid. There was significant difference in the fatty acid composition of broiler meats ($P < 0.05$) fed the experimental diets. The broiler fed with thevetia oil contained the highest alpha linolenic acid which is an n-3PUFA.

Keywords: meat, fatty acids, poly unsaturated, Thevetia, oil

S10- 0016 Application of isoelectric solubilization/precipitation processing to improve gelation properties of protein isolated from pale, soft, exudative (PSE)- like chicken breast meat

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Currently, some processors concerned about the appearance of pale, soft, exudative (PSE) cut-up chicken breast. The downgraded quality issues of meat protein, such as reduced water holding capacity and inferior texture, were resulted from protein denaturation as ultimate pH of PSE-like chicken breast was close to the isoelectric point of myofibrillar proteins. Isoelectric solubilization/precipitation (ISP) process has been attempted to improve gelation properties of PSE-like chicken breast meat protein. The recovered protein was recovered at pH values of 2.5, 3.0, 3.5, 11.0, 11.5 and 12.0. The results showed ISP treated with pH 11.0 and pH 3.5 significantly increased ($P < 0.05$) texture properties and water holding capacity (WHC). Among these conditions, whiteness of cooked sample was obviously enhanced ($P < 0.05$). SDS-PAGE indicated ISP process significantly affect salt extracted protein profile rather than that of total protein. ISP treatment altered the dynamic rheological behavior of PSE-like samples. Since enhanced texture was induced by ISP, the low elastic (G') modulus revealed ISP-extracted sample form more rigid gel instead of elastic one. Based on our study, the best gel properties were obtained by solubilizing the PSE-like chicken breast muscle at pH 11. In conclusion, this method may have potential to increase the economic value of PSE-like chicken breast meat by improving the protein gelation properties.

Keywords: isoelectric solubilization/precipitation, PSE-like, chicken breast meat, gelation properties

S10-0018 Effect of cold storage time on breast meat texture profile analyses of broiler chickens fed clinoptilolite coated with nanosilver particles

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The prospective of nano-technology in poultry meat industry cannot be fully appreciated yet because of lack of sufficient research and knowledge. Nanosilver and silver salts have currently been used in animal feeding as growth promoter and anti microbial agent in poultry industry is recommended with caution. The main purpose of this study was to investigate the effect of cold storage time on breast meat texture profile analyses of broiler chickens fed clinoptilolite coated with nanosilver particles. A total number of 375 one-day-old broilers (Ross 308) from a commercial hatchery were randomly assigned to 5 experimental groups in a completely randomized design (CRD). Experimental diets were following: 1) Basal diet (Control), 2) Basal diet supplemented with 1% clinoptilolite 3, 4 and 5) Basal diet supplemented with 1% clinoptilolite coated with 25, 50 and 75ppm nanosilver respectively. Three days after storage in the refrigerator, adhesiveness and Geminis were not influenced by dietary treatment ($P>0.05$) while hardness, springiness, chewiness and cohesiveness were affected most strongly by experimental diets ($P<0.05$). Seven days after storage of broiler chicken breast in the refrigerator only springiness and chewiness value were affected by the dietary treatments ($P>0.05$). The lowest value of springiness and chewiness were measured in the broilers fed 25 ppm nanosilver diet. Hardness, adhesiveness, cohesiveness and gumminess value were not influenced by treatment diets ($P>0.05$). It could be concluded that clinoptilolite coated with nanosilver could be used as growth and health promoters in the broiler diets without adverse effects on meat quality. So, it will need further work in the future.

Keywords: broiler, clinoptilolite, nanosilver, meat quality, texture profile analyse

S10- 0019 Comparative analysis of AA broilers and pheasants in slaughter performance and meat quality

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In this study, AA broilers and pheasants were selected as experimental materials and they were slaughtered for comparative slaughter performance and meat quality. 20 seven-week-old AA broilers and 20 seventeen-week-old pheasants were raised in the same environment and fed the same diet for two weeks, then they were slaughtered. The results showed that the abdominal fat rate of AA broilers was extremely significantly higher than pheasants ($P<0.001$). The muscle shear force of pheasants was extremely significantly higher than AA broilers ($P<0.001$). The breast muscle shear force of pheasants was 13.41 and the leg muscle shear force of pheasants was 15.02. The breast muscle shear force of AA broilers was 2.30 and the leg muscle shear force of AA broilers was 2.38. The muscle shear force of pheasants was 6 times as much as AA broilers. It showed pheasants are not suitable for frying technology, more suitable for processing stewed way which can reflect good cooking and chewiness; There were not significant difference in drip loss rate and cooking loss rate between AA broilers and pheasants ($P>0.05$). But the drip loss rate and cooking loss rate of pheasants were less than AA broilers. It showed that pheasants have advantage in the storage, transportation and processing.

Keywords: pheasant, AA broiler, slaughter performance, meat quality

S10- 0020 Risk assesment of heavy metal exposure through chicken meat consumption in and around Proddatur region of Andhra Pradesh, India

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Introduction: Andhra Pradesh is leading in Poultry industry. Consequently, there is an increase in the consumption of chicken meat. Recently, there is heightened consumer awareness regarding the presence of heavy metals in food products. Hence, in this study, we assessed the presence of heavy metals in chicken meat sold through retail shops in and around Proddatur region of Andhra Pradesh. This area is an industrial area with chemical, cement industries and thermal power station. **Material & Methods:** Samples of chicken metals were collected from different areas in and around Proddatur and were digested using nitric acid with microwave digestion system. The amount of heavy metals in the milk and water samples were estimated using ICP- OES. **Results:** The level (ppm) of various heavy metals in Chicken meat were: Arsenic (9.33 to 10.27); Lead (7.97 to 8.26); and cadmium (2.25 to 2.50). **Conclusion:** The area under the study falls in sedimentary rock area and hence, water and feed contain high level of heavy metals. We found that the level of heavy metals in chicken meat was higher than permissible limits and needs urgent attention.

Keywords: chicken, meat, lead, arsenic, cadmium

S10- 0021 Effects of pressure assisted thermal processing on water and texture characteristics of salt- reduced chicken sausages

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Reducing salt content in meat products has become increasingly necessary, as the relatively high amount of salt used in meat products contributes substantially to chronic health risk related to excessive salt intake. In this study, pressure assisted heating (PAH) is proposed to reduce salt content in meat products. Meat batters were prepared from fresh chicken breast meat (Pectoralis major M.) using various NaCl concentrations (0-2%), then filled into polyamide casings and subjected to PAH processing (up to 400 MPa for 30 min at 75° C). PAH-treated samples were then assessed for appearance, water loss, centrifugation loss, and hardness via texture profile analysis, water mobility and distribution were measured by low field-NMR T2 relaxometry, and microstructures were observed by scanning electron microscopy (SEM). Results showed that pressure has a dominant influence over salt on PAH-treated samples, while the effect of salt was more obvious among the samples heated under ambient pressure (H). There was no noticeable cooking loss in PAH-treated samples, while the cooking loss of H-treated samples increased significantly as salt content decreased. SEM results indicated that PAH processing contributed to disruption of myofibrils and formation of a fine strand gel network that could hold more water. The appearance and hardness as-observed indicated that protein gelation was promoted by 200 MPa PAH treatment, resulting in a rigid gel with low centrifugation loss. Low-field NMR further showed that the T21 and PT21 of 200 MPa PAH treated samples increased simultaneously, indicating that more water was immobilized. However, the samples treated with 400 MPa PAH showed a weak texture and high water loss, indicating that 400 MPa deteriorated the thermal gelation of the chicken batter. Hence, PAT when applied at proper pressure (around 200 MPa) can be used to reduce water loss and improve the textural properties of salt-reduced chicken sausages.

Keywords: high pressure, chicken meat, thermal, texture, water holding capacity

S10-0022 A rapid method for evaluating the freshness of chicken meat

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To explore the freshness of chicken meat and qualify the grade of meat products, we construct a rapid method for evaluating the freshness of chicken meat by using the ATP bioluminescence. Breast and thigh meat from 3 yellow chickens were frozen at minus 20 degrees for 45 minutes after slaughtered. Taking 0.5g breast meat and 0.5g thigh meat into homogeneous tubes with 4.5 ml sterile water at 4 hours post-slaughter (hps), 8hps, 12hps, 24hps, 48hps and 72hps, respectively, and we homogenized them and took the 10 μ l supernate after centrifugation onto the ATP testing swab. Finally, the number of moles of ATP in the breast and thigh meat were calculated through the ATP standard curve based the ATP bioluminescence assay. As a result, the content of ATP in both breast and thigh meat were decreased continuously to zero, but increased a little bit at 12hps, which corresponded to the ATP degradation rule basically. Combining the ATP content of the microorganism on the surface of the meat, we can evaluate the freshness level of meat rapidly. In conclusion, we confer the meat of first 4hps as the first freshness grade, the meat during 4hps to 12hps as the second freshness grade, the meat during 12 to 24 as the third freshness grade and the meat during 24 to 72 as the fourth freshness grade. At last the meat above 72h post-slaughter belong to non-freshness or frozen product.

Keywords: chicken meat, freshness, grade, evaluating method

S10-0023 Occurrence of breast meat abnormalities and foot pad dermatitis in fast-growing chicken hybrids

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A trial was conducted to compare productive performances, incidence of breast myopathies and foot pad dermatitis of two commercial fast-growing chicken hybrids (HA and HB) characterized by different growth profiles. A total of 1,920 one-day-old female chicks, obtained from the same hatching session, were housed in an environmental controlled poultry house, divided in 2 groups of 9 replications each and fed the same commercial diets. Productive parameters were recorded at 0, 9, 21 d and at slaughter (35 d). Breasts were classified according to the degree of white striping, wooden breast and poor cohesion defects (normal, moderate and severe lesions). Foot pad dermatitis were also scored according to the severity of lesion using a 3 points scale. At slaughter, no significant difference emerged in terms of productive performances albeit the two hybrids showed different growth patterns. Indeed, at 9 d, HB birds showed a lower BW than HA ones (216 vs 227 g, respectively; $P < 0.01$) while at 21 d they resulted heavier (775 vs 751 g, respectively; $P < 0.01$) and showed a better feed conversion rate (1.511 vs 1.632, respectively; $P < 0.05$). Overall, carcass yields were not modified. HB chickens showed higher percentages of breast without any signs of white striping (69 vs 39%; $P < 0.001$), wooden breast (75 vs 41%; $P < 0.001$) and poor cohesion (61 vs 37%; $P < 0.001$) if compared with HA birds. Finally, HB group had also a remarkably higher incidence of birds without foot pad dermatitis (53 vs 23%; $P < 0.001$). In conclusion, it emerged that broiler chickens belonging to two different genotypes, hatched and raised in the same environmental conditions and fed the same diets showed substantial different magnitude of either breast meat abnormalities or foot pad dermatitis although reached similar body weight at slaughter. These results highlight the importance of better understanding the effects of selection applied to fast-growing chicken hybrids on the onset of main meat and carcass defects.

Keywords: broiler chicken, fast-growing hybrids, productive performance, meat abnormalities, foot pad lesion.

S10- 0024 Partial or total replacement of soybean oil by black soldier pupae fat in broiler diets. Part 2: Effect on breast meat thawing loss, proximate composition and rheological properties during retail display

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The study tested the effect of a partial and total replacement of soybean oil with black soldier (*Hermetia illucens*) pupae fat (BSPF) on broiler meat quality. The meat derived from birds of three feeding groups: broilers of the first group received a basal diet based on corn meal, soybean meal and soybean oil (C), whereas in the other two groups the 50 (CH) and 100% (H) of soybean oil was replaced by BSPF. After slaughtering and dissection, a total of 48 breast muscles were halved in right and left side, and vacuum-packed. At the laboratory of the Department of Animal Medicine, Production and Health of the University of Padova, the right breasts were frozen at -40 °C whereas 24 hour post mortem the left breasts were freed from plastic bag, weighed and the pH was measured by infission in the cranial and caudal part of the Pectoralis major muscle. In the same sites, colour measurements (lightness L*, redness a* and yellowness b*) were performed (day 0). Afterwards, breasts were individually placed on polystyrene trays, wrapped with plastic film to minimize direct air contact, and stored at +4°C under fluorescent light illumination for a 9 days retail display. The same analyses conducted at day 0, were repeated at day 9 of storage and drip loss was also calculated. After fifteen days of frozen storage, right breasts were weighed, thawed for 12 hours at +4°C, and weighed again to compute thawing loss. After grinding at 7000 g for 10 seconds and freeze-drying, breasts were then analysed for proximate composition. Breast meat from C, CH and H dietary groups showed similar weight, thawing loss and proximate composition. Breast meat from C, CH and H feeding groups did not differ in pH and L*, a*, b* colour values, both at 0 and 9 days of retail display. Also drip loss did not differ among treatments. In conclusion, BSPF seems a promising ingredient in broiler diet as no adverse effect on the studied meat quality traits was observed.

Keywords: Alternative ingredients, insect fat, chicken meat, meat quality, retail display

S10-0025 Feasibility of functional fibers in soy chicken nuggets

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Soluble fiber has many health benefits. However most people have less than the recommended daily value. The study objective was to develop soy chicken nuggets with added functional fibers to increase dietary fiber intake. Soy chicken nuggets were made using an industry formulation of 30% pre-hydrated textured soy protein concentrate (3:1 wt/wt water:soy) with 10% brine resulting in 0.60% salt and 0.50% phosphate in the final product. Treated nuggets were formulated to add 3.0 g of functional fiber (70% isomalto-oligosaccharide (IMO), 30% hydroxypropyl methylcellulose (HPMC)) per serving (3 nuggets, 87g) based on the control formula. Nuggets were evaluated for pick-up %, par-fry yield %, cook loss %, frozen loss %, final yield %, color (L*, a*, b*), shear force and fiber content including total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). All data were analyzed using ANOVA (SAS 9.3) and significance was determined using a P < 0.05. Significant results indicate that fiber nuggets had lower par-fry yield (3.05 and 2.65), lower freeze loss (1.82 and 1.46), lower L* (78.29 and 76.97) indicating a darker color, higher b* (12.09 and 12.76) indicating more yellow, and were more tender (13.73N and 7.17N) when compared with controls. Fiber nuggets contained 2.91g TDF per serving, which is considered a "Good Source" of fiber, but the fiber results were much lower than the formula supplementation level by AOAC 985.25 and 991.43 and AOAC 2009.01 and 2011.25. This difference could be due to the inability to detect HPMC and IMO using these methods. In conclusion, functional fibers have the potential to be incorporated in the meat block formulation to produce fiber-added nuggets, but more reliable fiber methods need to be developed for determining the fiber contents in meat products.

Keywords: chicken nuggets, fiber, HPMC, IMO

S10-0026 Effects of qualitative nutrient allocation from 8 to 35 d of age on subsequent myopathies of the Pectoralis major muscles in broiler chickens

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Quantitative control of nutrient intake may decrease the incidence of breast muscle myopathies [white striping (WS), wooden breast (WB), and necrosis (N)] with some impairment in live performance at a given age. An experiment was conducted to determine if a similar reduction in myopathies may be obtained by altering dietary nutrient density. Male broiler chicks of a high-yielding commercial strain were placed into 63 pens (22 birds/pen). All birds received an identical prestarter diet until 7 d of age, after which time each pen was assigned to 1 of the following 7 dietary treatments (TRT; 9 replicate pens per TRT) for the starter (8 to 14 d), grower (15 to 25 d), and finisher (26 to 35 d) phases: 1) 100% of primary breeder recommendations for digestible lysine and apparent metabolizable energy density throughout experiment; 2) 95% of TRT 1 until 14 d of age, then as TRT 1; 3) 95% of TRT 1 until 25 d of age, then as TRT 1; 4) 95% of TRT 1 throughout experiment; 5) 90% of TRT 1 until 14 d of age, then as TRT 1; 6) 90% of TRT 1 until 25 d of age, then as TRT 1; 7) 90% of TRT 1 throughout experiment. Diets were formulated to maintain ideal amino acid ratios to digestible lysine. Blood samples from 4 birds per pen at 33 d of age were analyzed for plasma creatine kinase (CK) and lactate dehydrogenase (LDH). At 35 d of age, 18 birds per pen were processed and evaluated for WS, WB, and N. Reduced dietary density in the starter phase (TRT 2 and TRT 5) resulted in increased ($P \leq 0.05$) incidences of severe WB (32.9% and 34.7%) and N (13.8% and 11.5%) relative to TRT 1 (WB: 18.2%; N: 3.5%). Plasma CK and LDH increased ($P \leq 0.05$) with increasing scores for WB, WS, and N. These results indicate that reducing dietary nutrient density from 8 to 14 d may exacerbate fillet myopathies in broilers reared to 35 d of age. Qualitative nutrient allocation programs that might reduce the incidence of breast myopathies while maintaining optimal performance require further evaluation.

Keywords: broilers, lysine, energy, breast fillet, myopathy

S10-0027 Effect of frozen storage period on meat quality of Korean native duck meats frozen rapidly

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This study was carried out to investigate effect of frozen storage period on meat quality of Korean native ducks after rapid cooling (-50°C). The meats used were breast meats collected from 24 ducks that were fed for 0~8 wks (2.8 kg of live weight) by basal diet based on corn-soybean meal and slaughtered with dimethyl ether. Experiment was conducted with total 12 samples divided into 4 treatments (3 replications/treatment, 1 samples/replication, 2 birds/sample): C (control, not to freeze), T1 (1 month after rapid cooling), T2 (3 month after rapid cooling) and T3 (6 month after rapid cooling). There was no significant difference on lightness, moisture among four treatments. Also there were no meaningful changes on other fat acids and amino acids among treatments. In conclusion, this study demonstrated that meat characteristics of physicochemical composition, fat acids and amino acids were not affected much by frozen storage period.

Keywords: Korean native duck meat, frozen storage period, meat quality, rapid cooling

S10-0028 Effect of age on the occurrence of muscle fibre degeneration associated with myopathies in broiler chickens submitted to feed restriction

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To evaluate how the age affected the occurrence of muscle fibre degeneration (MFD) associated with white striping and wooden breast, Pectoralis major muscles of 192 broiler chickens differing for genotype (standard vs. high breast yield), gender, and feeding regime (ad libitum vs. restricted rate, 80% from 13 to 21 d of age) were sampled at different ages (14, 21, 28, 35 and 46 d) for histological analyses and using H&E staining to evaluate tissue general morphology, Masson's Trichrome to identify collagen presence, Oil red and Nile blue for lipid presence. The effect of age, genotype, gender and feeding regime on the frequency of chickens showing MFD was evaluated by PROC CATMOD of SAS. Thereafter, significant differences according to the feeding system within age were assessed by χ^2 test. On average (data of all slaughters), nor genotype (69.8% vs. 67.7% in standard vs. high breast yield) or gender (68.8% in males and females) affected the frequency of chickens with MFD. Differently, this latter frequency was higher with ad libitum feeding than early restriction (75.0% vs. 62.5%; $P=0.01$) and increased with age (18.8%, 28.1%, 75.1%, 96.9%, and 96.9% at 14, 21, 28, 35, and 46 d). In details, at 14 d a similar frequency was found (18.8%) in all broilers; at 21 d, at the end of the restriction, MFD occurred more in broilers fed ad libitum than in those under restriction (50.0% vs. 6.3%; $P<0.01$); at 28 d a similar trend was observed but differences between the two groups were reduced (87.5% vs. 62.5%; $P=0.10$) to disappear by the 35th (100% and 93.8%) and 46th d (96.9% and 96.9%). In conclusion, MFD soon occurred in broilers after two weeks of growth and increased dramatically within 28 d of age. Feed restriction was effective in controlling and reducing MFD occurrence only as long as animals were under restriction, but no residual positive effect was recorded after their re-alimentation.

Keywords: genotype, gender, histology

S10- 0030 Inhibition of Salmonella by cecal bacteria in media supplemented with lactate and succinate

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Experiments were conducted to examine the ability of cecal cultures from broilers to inhibit growth of *Salmonella Typhimurium* in vitro. Cecal contents from commercial broilers were combined, and 0.1 ml of the cecal slurry was added to media containing (g/l), tryptose, 10; yeast extract, 5; sodium chloride, 5; beef extract, 2; and glucose, ² Inoculated media was incubated aerobically at 37°C for 48 h. Supplemented media was prepared by adding 0, 50, 100, or 150 mM of sodium lactate and sodium succinate to fresh media. Supplemented media was inoculated with 105 cfu/ml of cecal culture, only; 104 cfu/ml of a nalidixic acid resistant *Salmonella*, only; or the cecal culture and *Salmonella*. Inoculated media were incubated aerobically at 37°C for 14 days. After incubation, *Salmonella* and cecal bacteria (aerobes and anaerobes) were enumerated in each media. Cecal bacteria were also isolated and then identified with the Biolog Microbial Identification System. Results indicated that there was no significant difference in the number of cecal bacteria recovered from any media inoculated with cecal cultures only or with cecal cultures and *Salmonella*. However, significantly ($P<0.05$) fewer *Salmonella* were recovered from media inoculated with cecal cultures and *Salmonella* than from media inoculated with *Salmonella* only in media supplemented with 50, 100, or 150 mM lactate and succinate. There was no significant difference in the number of *Salmonella* recovered from media that was not supplemented with lactate and succinate. Aerobic cecal isolates included *Enterococcus faecalis*, *Escherichia coli*, and *Corynebacterium amycolatum*, while anaerobic isolates included *Lactobacillus* spp., *Lactococcus* spp., and *Streptococcus* spp. Findings indicated that cecal cultures from broilers possess anti-*Salmonella* activity related to the ability of the cultures to metabolize lactate and succinate. This information may be beneficial in formulating effective, defined probiotic cultures.

Keywords: cecal cultures, competitive exclusion, *Salmonella*

S10- 0031 Effect of pH, water content, fat and protein in different commercial chicken brands available in the market of Saudi Arabia

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Three commercial brands of chicken available in the market produced from different poultry processing plants in the Kingdom of Saudi Arabia were considered for the study. From each brand, 6 samples with same production date were collected for studying the raw meat pH, water content, fat percentage and Protein content from each sample. pH was analyzed by using FT- NIR whereas water content, Fat and protein were analyzed by using standard methods. In whole chicken the pH values significantly ($P>0.05$) varied in brand 1 compared to brand 2 and 3. In case of breast fillet the pH values were significantly ($P>0.05$) higher in brand 1 & 2 when compared to brand 3. In breast fillet with skin and Drumsticks with skin the pH values significantly varied among all 3 brands. The Fat percentage in all the mixed portions including whole chicken varied significantly ($P>0.05$) in all brands except for wings with skin. Water content of breast fillet (86.83 ± 0.13 , 87.42 ± 0.05 and 85.07 ± 0.04 in Brand 1, 2 and 3 respectively), thigh cut skin on Bone (60.27 ± 0.08 , 56.12 ± 0.67 and 58.10 ± 0.38 in Brand 1, 2 and 3 respectively) and breast fillet with skin (56.08 ± 0.93 , 76.59 ± 0.32 and 79.87 ± 0.27 in Brand 1, 2 and 3 respectively) differed significantly ($P>0.05$) in all brands. However, the fat percentage of whole chicken and breast fillet with skin are higher in brand 1 when compared to brand 2 whereas fat percentage in breast fillet without skin is higher in brand 1 when compared to brand 3. The protein content of thigh cut, wings with skin and breast fillet with skin significantly differed among three brands. The results indicate that there are wide differences in raw meat composition in different portions of the chicken when compared to whole chicken with skin and without skin. Concluded that among the three brands analysed for various parameters the brand 2 is significantly better for consumer.

Keywords: broiler meat, pH, protein, water content and fat percentage of mixed portions

S10-0032 Muscle and serum profile of broilers as influenced by dietary inclusion of different oils

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The muscle and serum profile of broiler chicken due to dietary inclusion of combinations of different commonly available oils, viz., coconut oil, fish oil, groundnut oil and rice bran oil were studied by rearing two hundred, sex separated, day-old, commercial (Vencobb) broiler chicks belonging to single hatch in deep litter system. All the chicks were wing banded, weighed and randomly allotted into five treatment groups with five replicates of eight chicks each for a period of six weeks. The treatment groups consisted of T1 (Basal diet with Fish oil), T2 (Basal diet with Fish oil + Coconut oil), T3 (Basal diet with Fish oil + Groundnut oil), T4 (Basal diet with Fish oil + Rice bran oil), T5 (Basal diet with Coconut oil + Groundnut oil + Rice bran oil). The experimental feed was formulated according to the Vencobb standards for different treatment groups. All the diets were isocaloric and isonitrogenous. The oils viz., coconut oil, fish oil, groundnut oil and rice bran oil were included in the ration at 1 per cent, 2 per cent and 3 per cent level in pre-starter, starter and finisher diets, respectively. At the end of the experiment (42nd day), each five males and females, totally ten birds per treatment group were randomly picked up and slaughtered for studying muscle and serum characteristics. There were no significant differences on total breast and thigh muscle cholesterol content (mg %) among the treatment groups. Statistical analysis of data on serum total cholesterol and LDL cholesterol did not differ significantly between treatment groups at sixth week of age. Significantly ($P < 0.01$) highest triglyceride value (104.84 mg %) was recorded in T2 group and the lowest value was observed in T3 group (76.63 mg %). Hence, it is observed that groundnut oil combined with fish oil (T3) in the basal diet lowered the breast and thigh muscle cholesterol and serum cholesterol levels in broilers.

Keywords: muscle and serum profile, different oils, broiler ration

S10- 0033 Reducing the prevalence of Salmonella-infected poultry flocks by a surveillance- and- elimination strategy-the Danish success

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In response to the rising incidence of human salmonellosis in Denmark during the 1990s, The National Danish Salmonella Control Programme for broiler and table egg production was implemented in 1996. The programme forms part of the Danish Parliament and Government's general objective of improving the quality and safety of Danish food. The programme is accomplished in collaboration between the national authorities, the poultry industry, and the farmers associations. Today 20 years later, the results of the programme are obvious. Since the launch of the programme, the prevalence of flocks infected with Salmonella have decreased by more than 20 % to less than 0,6 % in both the table egg and the broiler sector. In 2012, an EU Salmonella guarantee for the Danish table egg production was achieved. In 2015, no infected table egg flocks were found, even though each flock was tested by sock samples every second week. For the broiler production, all broiler flocks are tested twice during production. The number of infected broiler flocks is decreasing, and in 2015 the prevalence was below 0,6 %. The programme is a 'top-down' surveillance-and- elimination strategy, whereby infected flocks are eradicated by means of compulsory destruction or slaughter. There is a zero tolerance for all Salmonella types in all poultry products in Denmark. Vaccination has never been used in the programme. The sampling programme includes both serological and bacteriological analyses. The programme also comprises code of practice guidelines, import restrictions of animal material, biosecurity guidelines, requirement for heat-treated feed, and control by national officials. Conclusion: It is possible to reduce the prevalence of Salmonella- infected poultry flocks by surveillance and elimination strategies. The total cost of the programme is more than 15 mill. €. Since 2002 all the expenses have been paid by the producers themselves.

Keywords: Salmonella, elimination, quality, food safety.

S10-0034 Comparing Salmonella Enteritidis and Salmonella Heidelberg colonization in broiler chickens that were continuously exposed to these bacteria in the feed

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Both Salmonella Enteritidis (SE) and Salmonella Heidelberg (SH) are important foodborne pathogens associated with poultry. This study was conducted to determine which organ(s) become colonized with these bacteria when the birds are continuously exposed to these bacteria in the feed. For this study, 500 straight run boiler chicks were obtained and randomly placed into 20 pens (10 pens/diet). Birds were given a standard diet that contained either 10x2-3 cfu/g of SE or SH. Both of the Salmonella's utilized were nalidixic acid and novobiocin resistant. On days 35-38 a total of seven birds were removed from each pen and necropsied. Samples collected include: cecal contents, breast muscle, thigh muscle, crop, kidney, pooled liver and spleen, pooled bursa and thymus, bone marrow, lung, trachea, skin, spinal cord and abdominal cavity swab. After harvesting, samples were immediately placed into BPW and stored at 4C until all the birds were necropsied after which, all the samples were incubated at 37C for 24 hours. From each BPW sample a subsample was taken and used to inoculate TTB tubes. The TTB tubes were incubated for 24 hours at 41C. After that time samples were plated onto XLT4 agar that contained nalidixic acid and novobiocin. These plates were incubated at 37C for 48 hours after which the plates were removed and Salmonella colonies enumerated. Results of this trial had shown at least one sample was positive for Salmonella in all the birds. There were differences between SE (41.4%) and SH (20.0%) for kidney, cloaca (SE=87.1%, SH=55.7%) and abdominal cavity (SE=44.3%, SH=27.1%). In addition SE colonized more sampled tissues (22.9%) then did SH (11.4%). Differences in colonization may be attributed to differences in virulence of the isolates used in this study. Regardless, the results of this study has shown that if birds are continuously exposed to Salmonella in the feed there is a 100% chance the birds will be positive for the bacteria.

Keywords: broiler, Salmonella, foodsafety, tissue

S10- 0036 Effect of White Striping and Wooden Breast occurrence on broiler quail and slow-growing chicken carcass and meat quality

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This study was the first to investigate occurrence of White Striping (WS) and Wooden Breast (WB) myopathies in breasts of broiler quail (*Coturnix coturnix japonica*) and slow-growing chickens and their effects on carcass and meat quality. To this end, N=180 28 day-old quails and N=119 180 day-old Padovana and Polverara breed chickens of both sexes were randomly selected in two commercial farms just before slaughter and weighed (SW). Chilled carcasses (CW) were weighed and checked for breast myopathies. Breast muscles were dissected from carcasses of both species and weighed, and yields to CW were computed. L*a*b* colour and pHu were measured in Pectoralis major muscles. Quail breasts were then vacuum-sealed, cooked in 80 °C water bath to 74 °C core temperature, cooled, dried, and weighed to determine cooking loss. Warner-Bratzler shear force was assessed on 4 cooked meat replicates per sample. A one-way ANOVA tested effects of the myopathies on the variables studied with SW as covariate. Linear regression between breast myopathies and physical traits was also performed. Myopathies (only WB defect was observed) affected 16% of quail breasts (n=29): WB lowered meat pHu and increased cooking loss compared to normal breasts (P<0.05). Regression coefficients were significant (P<0.05) for pH (-0.06 pH units) and cooking loss (2.2%). Myopathies afflicted 23% of chicken breasts (WB: n=8; WS: n=11; WSWB: n=3) without affecting physical traits. In chickens, the regression coefficient was significant with WS for SW (+262 g), carcass yield (+1.65%), and L*, a*, b* (-1.95, +0.75 and -1.93 colour units, respectively), and with WSWB for SW (+450 g), breast weight (+55.3 g) and b* value (-3.04 colour units). In conclusion, this study showed that WS and WB myopathies occur also in broiler quails and slow-growing chickens, and that SW seems to play a key role in myopathy development.

Keywords: meat quality, Japanese quail, slow-growing chicken, wooden breast

S10-0037 New insights on functionality of white-striped and wooden broiler breast meat

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In the past few years, a remarkable increase in the incidence of breast muscle abnormalities termed as White- Striping (WS) and Wooden- Breast (WB) (normally downgraded by the poultry industry and used as raw materials to produce processed products) was observed. Thus, a study was conducted in order to investigate the implications of WS and/or WB abnormalities on fatty acid composition, lipid and protein oxidation and physico-chemical state of water assessed by time domain nuclear magnetic resonance. Twenty boneless and skinless Pectoralis major muscles were selected from the same flock of high-breast yield hybrids (males) in the deboning area of a commercial processing plant and subsequently graded by visual appearance and manual palpation according to the presence of WS and WB conditions and divided in four groups: 5 Normal (NORM), 5 WS, 5 WB and 5 WS/WB samples. At 24 hours post-mortem, a sample obtained by each fillet was used to determine the NMR T2 relaxation time, while the residual part was finely minced and used to assess composition fatty acid profile and total heme content as well as lipid and protein oxidation. Overall, negligible modifications in fatty acid profile were found. In comparison with NORM, an overall decrease (P<0.001) was found in WS, WB and WS/WB samples concerning the total amount of heme pigments. Otherwise, even if no significant differences were found in WS and WS/WB samples, the WB group exhibited higher (P<0.05) TBARS value in comparison with NORM (0.41 vs. 0.22 mg MDA/kg of meat). Moreover, higher carbonyls were measured in WB and WS/WB samples than in NORM and WS ones (1.11 and 1.12 vs. 1.35 and 1.28 nmol/mg of protein; P<0.001). Abnormal samples showed a remarkable increase in proportion and mobility of extra- myofibrillar water fraction especially in WB and WS/WB groups. As a result, both functional and quality issues might arise when processed products are formulated including raw meats affected by WS and/or WB.

Keywords: breast meat, white striping, wooden breast, oxidation, NMR, fatty acid composition

S10-0038 Identification and biocide susceptibility of dominant microbiota after cleaning and after disinfection of broiler houses

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Hygiene in animal production is key for both farm management and food safety. Cleaning and disinfection of broiler houses is essential to manage farm hygiene. Mean total aerobic flora count is still 5.7 and 4.2 log CFU/625 cm² after cleaning (AC) and after disinfection (AD) of broiler houses, respectively. However, nothing is known about the microbial composition in the environment AC and AD. In addition, the question why some bacterial species/strains are still present AD whereas other are killed after C&D remains. The study was carried out in 4 broiler houses. Sampling was performed AC and AD. The disinfection product was based on hydrogen peroxide and peracetic acid. Enumerations AC and AD were carried out for total aerobic flora, Enterococcus spp. and Enterobacteriaceae. The dominant microbiota present was assessed by (GTG)5 analysis and 16S rRNA gene sequence analysis. In addition minimum biocide concentration (MBC) tests were carried out on selected isolates. In total, 363 and 255 isolates were identified AC and AD, respectively. The most dominant genera found were Brevibacterium, Brachybacterium and Staphylococcus AC and Bacillus, Brevibacterium and Staphylococcus AD. On both sampling moments Enterococcus faecium was mostly isolated as Enterococcus sp.. Of the Enterobacteriaceae medium, genera Enterobacter and Pantoea; and Aeromonas (non Enterobacteriaceae) were most abundant AC and Escherichia, Lelliotta and Pantoea AD. Per sampling point, 4 to 9 different genera were found AC, and 6 to 12 different genera AD. Per type of sampling point (drain hole, floor, floor cracks, drinking cups, walls, ...) differences in dominating flora were found. MBC results show no selection to less sensitive bacterial strains for the used biocide AD compared to AC. Enterobacteriaceae strains isolated AD (mean MBC: 0.8%) were slightly less susceptible than strains isolated AC (mean MBC: 0.7%). However, the opposite was observed with Enterococcus faecium strains.

Keywords: cleaning and disinfection, microbiota identification, biocide susceptibility, broiler houses

S10-0039 Effects of pressure assisted heating on the gel properties of salt-reduced chicken sausage batters

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Reducing salt in meat products over the years has become increasingly necessary, due to the relatively high amount of salt used in meat products contributes to chronic health risk that are related to high-salt intake. In this study, pressure assisted heating (PAH) was proposed to reduce salt in chicken meat products. Meat batters were prepared from fresh chicken breast meat (Pectoralis major M.) using various NaCl concentrations (0-2%), then filled into polyamide casings and subjected to PAH processing (up to 400 MPa for 30 min at 75°C). PAH-treated samples were then assessed for appearance, water holding capacity and hardness using texture profile analysis. Water mobility and distribution were measured by low field-NMR T2 relaxometry, and microstructures were observed by scanning electron microscopy (SEM). The effect of PAH treatment on physical properties of chicken sausages was affected by salt concentration and particularly by pressure level. The application of PAH treatment at 200 MPa improved the low-salt sausage qualities as compared to non-PAH treated samples, while a deterioration effect was observed at 400 MPa. The appearance and hardness as observed indicated that protein gelation was promoted by PAH treatment at 200 MPa, resulting in a rigid gel with low centrifugation loss. Low-field NMR showed that the T21 and PT21 of 200 MPa treated samples increased simultaneously, indicating that more water was immobilized. SEM results further revealed that high pressure contributed to disruption of myofibrils and formation of a fine strand gel network. The results demonstrated that pressure assisted heating can be used to improve physical properties of salt-reduced chicken sausages.

Keywords: high pressure, chicken meat, thermal, texture, water holding capacity

S10-0040 The role of the microbial monitoring in the prevention of poultry bacterial infections

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Resistance to antibiotics is a problem for most countries of the world. The infections are caused by resistant strains of Salmonella, E.coli and Campylobacter cause serious diseases and can be lethal for animals and humans. The aim of research was to monitor microorganisms circulating in poultry farms of Ukraine, sensitivity to antimicrobial drugs and to develop system of rotation for prevention of poultry's bacteriosis. It was done in lab of Sumy National Agrarian University and Ukraine's poultry farms. Microbiological monitoring was carried out with using R-biopharm's test systems. The sensitivity of isolated pathogens to antimicrobial agents was tested by serial dilutions. We found that respiratory syndrome is caused by *S. aureus*, *S. pneumoniae*, *C. perfringens*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *P. vulgaris*, *S. enteritidis*, *M. gallisepticum*, *P. multocida*, *A. fumigatus*.. The intestinal syndrome is caused by *S. aureus*, *S. faecalis*, *C. fetus*, *C. jejuni*, *C. perfringens*, *E. agglomerans*, *E. coli*, *P. aeruginosa*, *P. vulgaris*, *S. enteritidis*, *S. pullorum-gallinarum*, *Y. enterocolitica*.. *E. coli* were represented as O2; O4, O8; O78, O157. Salmonellas were identified as: *S. enteritidis*, *S. typhimurium*, *S. pullorum*, *S. gallinarum*, *S. virchow*, *S. infantis*, *S. arizonae*, *S. jawa*, *S. montevideo*, *S. copengagen*. Bactericidal activity to the isolated cultures had apramycin, enrofloxacin, colistin, polymyxin, trimethoprim, tylosin, tiamulin, sulfadiazine. *P. aeruginosa* was highly sensitive to apramycin, tylosin and polymyxin. *S. aureus*, *S. pullorum*, *C. jejuni*, *E. coli* O2 were sensitive according to quinolones and cephalosporins. According the results we developed antimicrobial schemes for rotation, control and prevention poultry's bacterial diseases.

Keywords: microbiological monitoring, prevention, rotation

S10-0041 Microstructure and quality of chicken meatball formed manually and with devices

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The experiment was conducted to investigate the microstructure and quality of chicken meatball formed manually and with devices. The meatballs were made of chicken meat, tapioca flour, garlic, pepper, salt, flavor enhancer and ice cubes. All ingredients were mixed and minced in a bowl chopper until homogenous dough was achieved. The meatball dough was formed manually and using forming devices and put in the hot water to cook. The chicken meatball were analysed for their microstructure and quality. The variables observed were microstructure, the chemical composition (moisture, protein, fat and ash contents), physical (water holding capacity/WHC, pH and tenderness) and sensory (color, taste, smell, texture, firmness, acceptability) characteristics of chicken meatballs. The microstructure of chicken meatball was descriptively analysed. The data of chemical composition and physical characteristics were analysed using analysis of independent T-test, whereas the sensory characteristics was analysed using analysis non parametric of Kruskal Wallis test. The results of the experiment showed that the chicken meatballs manually formed were more compact than the chicken meatballs formed using the device. There were not any different in chemical composition of all chicken meatball except the fat content. Manual chicken meatballs contained higher fat content (6.09%) than the device formed chicken meatballs (5.30%). The WHC and flavor of manual chicken meatballs were lower than the device formed chicken meatballs. It could be concluded that manual chicken meatball had more compact microstructure and more firmness meatballs than the devices ones but there were not any different in the characteristics of chicken meatballs.

Keywords: microstructure, quality, chicken meatball, forming device

S10- 0042 Effect of wooden breast condition on water–holding capacity of broiler breast pectoralis major

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The wooden breast condition (WBC) is an emerging muscle abnormality impacting broiler breast meat. Limited studies have shown that WBC may adversely affect meat quality. The objective of this study was to evaluate the effects of WBC on water-holding capacity (WHC) of broiler breast fillets (pectoralis major). Broiler breast fillets deboned at 3 h postmortem were collected from a commercial plant and categorized as normal, moderate, or severe WBC based on the incidence and severity of diffuse hardened areas throughout the fillets and the degree of palpable rigidity. Meat WHC was evaluated by four different methods: purge loss, salt-induced water uptake, thaw loss, and cook yield. Results showed that there was no difference in average purge loss (%) between the normal (1.05%) and severe WBC (1.16%) fillets. However, salt-induced water uptake, thaw loss, and cook yield values of breast meat with WBC were lower than normal fillets by approximately 15%, 1%, and 7%, respectively ($P < 0.05$). There were no differences in salt-induced water uptake, thaw loss, and cook yield between fillets with moderate and severe WBC ($P > 0.05$). Data demonstrate that the effects of WBC on meat WHC vary by measurement method. These results indicate that WBC may not influence the amount of purge accumulation in packages of fresh or frozen/thawed fillets. However, WBC may negatively impact meat marination performance and the yield of cooked products.

Keywords: broiler, breast meat, wooden breast condition, water-holding capacity

S10-0043 The physico-sensory characteristics and β -carotene content of carrot-supplemented chicken nugget kept in the refrigerator

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The experiment was conducted to investigate the physico-sensory characteristics and β -carotene content of carrot-supplemented chicken nugget kept in the refrigerator. Chicken nuggets were made of boned breast broiler chicken, ground fresh-carrot, garlic powder, pepper, salt, pala powder, chicken extract block, wheat flour, tapioca flour and chicken eggs. The amount of carrot added were 0, 5, 10 and 15% of the nugget dough. All ingredients excluding batter and bread crumb were mixed in a chopper, and then they were moulded, battered, enrobed with bread crumb and fried. Chicken nuggets were kept in a refrigerator for 0, 2, 4, and 6 days. The samples of chicken nuggets were taken out of the refrigerator for analysis consisting of their physical (pH, water holding capacity/WHC, firmness) and sensory (color, flavor, texture, firmness, and acceptability) characteristics as well as their β -carotene contents. The data collected were analysed using analysis of variance (factorial 4×4) for the physical characteristics and β -carotene content, whereas the data of sensory characteristics were analysed using non-parametric analyses with Kruskal-Wallis Hedonic test. Any significant different of means were further-tested using Duncan's new Multiple Range Test. The results showed that carrot supplementation significantly decreased pH, WHC, firmness, and flavor of chicken nuggets, however, it increased color and β -carotene content of chicken nugget ($P < 0.05$). Storage time significantly decreased pH, WHC and firmness and β -carotene content of chicken nuggets ($P < 0.05$). The average β -carotene content of supplemented chicken nuggets was 2,769.8 $\mu\text{g}/100\text{ g}$ chicken nuggets. It decreased during storage time to become averagely 1,543.91 $\mu\text{g}/100\text{ g}$ chicken nuggets at the end of experiment. It could be concluded that carrot supplementation and storage time affected the physico-sensory and β -carotene content of chicken nuggets.

Keywords: physico-sensory characteristics, β -carotene, carrot, chicken nugget

S10-0044 Four month shelf-life at 4±1 °C for sous vide processed chicken sausages

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Processing of sous vide chicken sausages was optimized under vacuum packaging condition and cooking at 100 °C for 30 min (SV30), 60 min (SV60) and 120 min (SV120) and compared with aerobically cooked control at 100 °C for 30 min. Sous vide processing of chicken sausages (SV30) produced higher ($P < 0.05$) cooking yield, Hunterlab a^* values and sensory attributes without affecting proximate composition and shear force values relative to control. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), 2-dimensional gel electrophoresis (2-DE) and scanning electron microscopy (SEM) results revealed no significant changes in protein quality/proteome character and emulsion ultrastructure due to SV30 processing relative to control sausages. Sous vide processing of chicken sausages enriched with rosemary diterpene phenols retained the freshness and quality up to 120 days during storage at 4±1 °C relative to control sausages that were spoiled on 20th day. Lipid oxidation and microbial growth remained below the spoilage levels for all the SV processed sausages throughout the storage and addition of rosemary diterpene mixture at 0.02% v/w reduced the microbial growth and improved ($P < 0.05$) the sensory attributes. Our results demonstrate that sous vide processing minimizes lipid oxidation and microbial growth of chicken sausages with improved product quality and shelf-life at 4±1°C. As the refrigerated meat products command premium price over frozen foods, the sous vide technology may be commercially exploited to better market emulsion chicken sausages under chilled conditions with a shelf-life of more than 4 months.

Keywords: sous vide, chicken sausages, rosemary diterpenes, ultrastructure, shelf-life

S10- 0046 Correlations between breast meat yield, quality parameters, color, woody breast and white striping

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Two common myopathies in broilers are white striping and woody breast which cause changes in meat color and quality. Myopathy scores are subjective and rapid objective measurements are necessary to characterize changes in meat quality. This experiment was conducted to evaluate the relationship between woody breast and white striping scores, relative Pectoralis minor and major weight, cook loss and drip loss on breast meat color. Broilers of 4 genetic lines (Athens - Canadian Random Breed Cross, Cobb 500 x Cobb, Ross 708 x Ross and Cobb x Hubbard M99) incubated under similar conditions were raised sex-separately in 80 floor pens and fed common diets ad libitum. At 57d of age, 20 males and 20 females from each line (2 per pen) were processed, cut up parts weighed, and myopathies scored 16 hours after deboning. Pectoralis major and P. minor from each bird were separated and color values of lightness (L^*), redness (a^*), and yellowness (b^*) were measured 24 h after processing using a Minolta colorimeter CR-400. Pairwise correlations were determined using JMP 11. The L^* value was positively ($P < 0.05$) correlated with relative P. major weight ($r = 0.80$), relative P. minor weight ($r = 0.57$), cook loss of P. major ($r = 0.40$), and white striping ($r = 0.32$). The b^* value was positively ($P < 0.05$) correlated with relative P. major weight ($r = 0.75$), relative P. minor weight ($r = 0.64$), woody breast scores ($r = 0.23$), cook loss of P. major ($r = 0.23$), white striping ($r = 0.17$) and drip loss ($r = 0.16$). The a^* value was positively ($P < 0.05$) correlated with woody breast ($r = 0.39$), white striping ($r = 0.38$), relative weight of P. major ($r = 0.27$) and cook loss on P. minor ($r = 0.27$). On the other hand, drip loss was negatively ($P < 0.05$) correlated with white striping ($r = -0.21$). In conclusion, myopathies and breast meat quality traits could be partially predicted by changes in breast color and the three dimensions of color should be used for these predictions.

Keywords: broiler breast meat color, woody breast, white striping, pectoralis major, pectoralis minor

S10-0047 Effects of genetic line, incubation temperature profiles and gender on woody breast and white striping at 57 d of age

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White striping and wooden breast are becoming common myopathies in heavy broilers worldwide. This experiment was conducted to evaluate the effects of genetic lines, incubation temperature profiles and gender on these myopathies. A total of 1,000 eggs from 3 genetic lines: Cobb 500 x Cobb MX, Ross 708 x Ross and Cobb x Hubbard M99, were randomly distributed into four machines with two incubation temperature profiles. In two machines, Standard eggshell temperatures were maintained close to 37.8°C during the whole incubation period to simulate single stage incubation. In the other two machines, eggshell temperatures were low (36.9°C) for the first 3 days and close to 37.8°C until the last 3 days when eggs were subjected to elevated (38.9°C) eggshell temperatures, as it is observed in multistage machines. Eggshell temperatures were measured with pipe-probes and thermistors. At hatch, 960 chicks (12/pen) were randomly distributed into 80 pens with 5 replicate pens/treatment combination. At 57 days, 2 birds per pen were processed and woody breast and white striping were scored 16 hours after deboning. Data were analyzed as a randomized complete block design with genetic lines, incubation profiles and sex as main effects. No ($P > 0.05$) three-way interactions were detected. Genetic by incubation temperature profile and genetic by gender interaction effects on white striping ($P < 0.05$) and differences due to genetics ($P < 0.001$) on wooden breast were observed. The most severe scores for woody breast and white striping were observed in Ross broilers and the lowest score for white striping was observed on Cobb x Hubbard males when these eggs were incubated to obtain 37.8°C of eggshell temperature during incubation period. Similar response was observed for Cobb x Hubbard females. In conclusion, genetic lines differed on white striping and wooden breast severity. Incubation temperature profiles may influence incidence of myopathies and those effects depend on genetic line.

Keywords: woody breast, white striping, incubation temperatures, genetic line

S10-0048 Gel properties of chicken salt-soluble proteins influenced by various types of peanut protein

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Salt-soluble proteins (SSP) play a significant role in producing chicken products with desirable texture and water-holding. The functional properties of poultry SSP were influenced by various factors, including pH, heating conditions, salt concentration and fiber type. Recently, application of non-meat proteins (soy protein isolate, wheat proteins, et al.) to modify the textural characteristics of poultry SSP gel becomes more and more prevalent. Peanut protein (PP) may be used in meat products to supply proteins and as a key ingredient. However, studies on the application of PP on meat, particularly chicken proteins are limited. To investigate the effects of PP on the gel properties of chicken during heat-induced gelation, we added various types of PP including defatted peanut flour (DPF), peanut protein concentrate (PPC), peanut protein isolate (PPI) to chicken SSP prepared from breast muscle and determined the gel properties. The addition of DPF, PPC and PPI all significantly increased water-holding capacity and gel strength of heat-induced chicken SSP gel ($P < 0.05$). At the same addition level, PPI was the best ($P < 0.05$) for increasing water-holding capacity and PPC was the best ($P < 0.05$) for increasing the gel strength of heat-induced chicken SSP gel. The gel strength of chicken SSP increased with increasing amount of PPC up to 2.5% where a maximum strength value was obtained. Differential scanning calorimetry (DSC) showed that the addition of DPF, PPC and PPI all increased ($P < 0.05$) transition temperatures (T_{max}) and enthalpy of denaturation (ΔH) of chicken SSP. Scanning electron microscopy (SEM) indicated that the chicken SSP ultra-structure was more uniform compact and homogeneous when PPI was incorporated than adding DPF and PPC. The results suggested that PP may be a potential protein additive for improving the properties of SSP gelations.

Keywords: chicken, peanut protein, salt-soluble proteins, heat-induced gelation

S10-0050 Effects of natural antioxidants on poultry and poultry products

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Antioxidants are substances that added to food products to minimize lipid oxidation, prevent development of off-flavors, and improve color stability. Nowadays, there is an increasing consumer demand on natural foods, which lead to processors to use natural antioxidants instead of synthetic antioxidants in foods. Poultry and poultry products have an important part in human diet, but the oxidative deterioration of lipids in poultry results in negative effects on sensory properties by rancid odors and flavors as well as nutritional properties by the formation of secondary compounds. The majority of currently used antioxidants in meat products are synthetic antioxidants. Thus, substitution of synthetic antioxidants with natural antioxidants have been gained most attention to retard lipid oxidation in poultry and poultry products in order to improve their quality and nutritional value. Recently, a great number of researches have been investigated the utility of natural antioxidants from several fruits and vegetable sources in meat products. The latest natural antioxidants used in poultry and poultry products are grape seed extract, green cabbage, cloves, rosemary, chestnut inner shell extract, green tea, plum, pomegranate, cinnamon, and berry extracts. Some studies have indicated that quality characteristics and shelf-life of poultry products such as chicken meatballs, chicken burgers, and chicken sausage can be improved by using natural antioxidants in some steps of processing. The major contributions of these compounds are diminishing lipid oxidation and microbial growth during storage. This review summarizes the recent information about potentially safe natural antioxidants which are currently used in poultry and poultry products. It also discusses the adequacy of antioxidants in poultry and poultry products.

Keywords: natural antioxidants, poultry products, oxidation

S10-0051 The studies on the change rule of IMP content of cold fresh chicken in different cold storage time

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Introduction: In order to provide the best storage time for improving the chicken flavored, and offer the necessary theoretical basis for the development and utilization of the cold fresh chicken product. **Materials and Methods:** 42 yellow feather broilers (21 hens and 21 cocks) feeding in the same condition in 70 days old were selected randomly. After slaughter and disinfection, cooled to 0~4°C quickly, and then conserved at 4°C freezer. Taken 3 hens and 3 cocks in the zero day (about 4 hours after slaughter), first day, second day, third day, fourth day, fifth day and sixth day respectively, gathered chest muscle, measured IMP, ADP, AMP, HxR, Hx and IMPc contents and studied the metabolic rule. The data were processed by SPSS 16.0 and Excel. **Result:** The results showed that, the IMP content in 0~6 days were 2.13mg/g, 1.90 mg/g, 1.40mg/g, 1.25 mg/g, 1.12 mg/g, 0.96mg/g, 0.92mg/g respectively, degradation tendency was obvious. The ADP content was highest in the third day (0.30mg/g) and lowest in the sixth day (almost complete degradation). The HxR content gradually increased to the fourth day (0.85mg/g), and then gradually reduced. The AMP and Hx contents were all reaching maximum in the sixth day. The IMPc content in 0~6 day were 3.18mg/g, 3.03mg/g, 2.88mg/g, 2.86mg/g, 2.90mg/g, 2.73mg/g, and 2.65mg/g respectively, the degradation tendency was obvious from the fifth day. **Discussion:** In the metabolic pathways of ATP to IMP, the storage temperature and times play an important effect on the IMP concentration. In this study, chicken were conserved at the condition of 0~4°C and measured seventh-consecutive day. The IMP content in the second day, third day and sixth day were the 70.0%, 56.3% and 43.3% of the 4 hours after slaughter respectively. It is suggested that when used household refrigerator to cold storage chicken, the storage time should be best control in 3days.

Keywords: cold fresh chicken, IMPc, IMP, change rule, cold storage time

S10- 0052 Evaluation of potential spoilage organisms isolated from chilled yellow-feather chicken in vitro and in situ

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Microorganisms play an important role in the spoilage of chilled chicken. A total of 53 isolates, belonging to 7 species of 3 genera, were isolated by a selective medium based on the ability to spoil chicken-juice. Four isolates of *Aeromonas salmonicida* 35, *Pseudomonas fluorescens* H5, *Pseudomonas fragi* H8 and *Serratia liquefaciens* 17 were further characterized for their proteolytic activities in vitro on meat proteins extracts and evaluated the spoilage potential in situ. In vitro study showed that 35 displayed the strongest proteolytic activity in both sarcoplasmic and myofibrillar proteins. However, the major spoilage isolate in situ was H8, which exhibited a fast growth rate, formation of slime, increased pH value and total volatile basic nitrogen (TVBN). The relative amounts of volatile organic compounds (VOCs) originated from microbiology, including alcohols, aldehydes, ketones and some sulphur compounds, increased during the storage. This study demonstrated the characteristic of 4 potential spoilage bacteria of chilled yellow-feather chicken and provides a simple and convenient method to assess the spoilage bacteria in the quality management.

Keywords: yellow-feather chicken, selective medium, spoilage bacteria, sensory analysis, volatile organic compounds.

S10- 0053 The correlation between polydactyly phenotype and product quality of Beijing fatty chickens

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Egg laying/meat dual-purpose type Beijing fatty chickens were selected and divided into three groups (bilateral four-toed, unilateral five-toed and bilateral five-toed) with four replications, 60 chickens per replications, 240 chickens per group. The production performance (weight, laying rate and egg weight), egg quality (Haugh unit) and muscle quality (pH, water-holding capacity, Warner-Bratzler shear force, TPA, nucleotide metabolites) characteristics of Beijing fatty chickens were determined. [Result] The weight of 120-d-old and 240-d-old bilateral five-toed Beijing fatty chickens was slightly higher than that of bilateral four-toed and unilateral five-toed Beijing fatty chickens, with no significant difference ($P>0.05$); egg weight of 160-d-old bilateral five-toed Beijing fatty chickens was significantly higher than that of bilateral four-toed and unilateral five-toed individuals ($P<0.05$). Water-holding capacity of leg muscles of bilateral five-toed Beijing fatty chickens was significantly superior to that of unilateral five-toed and bilateral four-toed individuals ($P<0.05$). The hardness, gumminess and chewiness of leg muscles of bilateral five-toed Beijing fatty chickens were significantly lower than that of unilateral five-toed and bilateral four-toed individuals ($P<0.05$), but the coherence and elasticity were remarkably higher compared with unilateral five-toed and bilateral four-toed individuals ($P<0.05$). Warner-Bratzler shear force of leg muscles of bilateral five-toed Beijing fatty chickens was significantly higher than that of other individuals ($P<0.05$). There was no significant difference in flavor substances among Beijing fatty chickens with different polydactyly phenotypes ($P>0.05$).

Keywords: Beijing fatty chicken, polydactyly, product quality

S10- 0054 Non- destructive prediction of pH value of fresh broiler chicken by visible near-infrared hyperspectral imaging

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VIS/NIR hyperspectral imaging was used to evaluate pH of chicken in this paper. Hyperspectral images (400- 1000nm) were acquired for bone side of chicken samples. In order to acquire the reflective spectral information from each single sample region, principle component analysis (PCA) and band thresholds on PC1 were used to eliminate the influence of background and undesirable pixels caused by reflection. Three pretreatment algorithms including Savitzky- Golay smooth, standard normal variate (SNV) and multiplicative scatter correction (MSC) were compared, and then partial least square regression (PLSR) was used to establish the predicting model. The optimized PLSR model has good performance with pretreatment of MSC. The coefficient of determination (R^2) were 0.99, standard error of prediction (SEP) were 0.0177. The results showed that VIS/NIR hyperspectral imaging could be used to predict the pH value of fresh broiler chicken.

Keywords: chicken, hyperspectral imaging, pH, PLSR

S10-0055 The study of meat quality changing regularity in chilled chicken meat and freeze- thaw chicken meat

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Significance: Chicken meat makes a significant contribution in our daily life as its overall nutrition and cheap price. The outbreak of bird flu makes traditional consumption habits have a change from hot fresh meat to chilled meat. The chilled meat has some advantages in comparison to hot fresh meat and frozen meat, such as more health and safety, tastier, and easier cooking. But now, as the chilled meat in market has no quality guarantee, customers always identify meat freshness and health through the colors, textures and smells. And these indicators are easy to be confused. There is no a quantitative method to assess the freshness and safety of meat. **Purposes:** Our research is in order to give some academic evidence of storage and cooking through testing the changing regularities of physical and chemical indicators of chilled meat and freeze-thaw meat. **Methods:** Altogether 71 yellow-feathered broilers aged 70d were sacrificed modeling on the slaughterhouse. Then we divided these chickens into two groups. The one group was chilled in 4°C for 7days, the another one group was frozen in -18°C for 24 hours and thawed in 4°C for 24 hours, and to repeat this cycle for 6. Every day we random selected 5 chilled meat and every cycle we random selected 6 freeze-thaw meat to continuous measurement their moisture content, lactic acid, inosinic acid, water-holding capacity, meat color, pH value and shearing force. **Results:** In a set amount of time, in chilled meat and freeze-thaw meat, the moisture content had no significant change; lactic acid content offered upgrade firstly than descending latter tendency, and freeze-thaw cycle could speed up the whole change process; the inosinic acid, pH value and water-holding capacity were all descended with time; the shearing force had no a obvious changing rules. The meat color in chilled meat had no marked variation, but the freeze-thaw meat changed more red and yellow.

Keywords: chilled chicken meat, freeze- thaw chicken meat, meat quality, physical and chemical indicators, Change regularity

S10-0056 Effect of feeding a combination of organic acids and mono-glycerides at different dosages and a probiotic on *Campylobacter* colonization in broilers

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An experiment was conducted within the EU-FP7 project CAMPYBRO in order to evaluate the appropriate dosage of a combination of a blend of mono-glycerides and organic acids (MGOA) and a probiotic (*B. subtilis* C-3102, P) on *C. jejuni* counts in broilers. There were four treatments applied from 1-42d of age: Control diet (C) and three diets based on C+MGOA (0.5% from 1-10d)+P (0.01%). The difference between the three treatments was the dose of MGOA from 10-42d, 1%, 2% and 3%, respectively. At 14d, all broilers (218 one-d-old Ross 308 broilers, male&female, 50%) were orally gavaged with 100 µl of a solution containing 1x10⁵ CFU/ml of ST-45 and ST-21 *C. jejuni* strains. At 21, 35 and 42d, ceca from 16 birds per treatment were collected and *C. jejuni* counts determined (ISO 10272). Data expressed as log₁₀CFU/g ceca content were first tested for normality and then analysed by the nonparametric test of Kruskal-Wallis (SPSS v.19.0). No significant differences between treatments were observed in performance for any of the periods studied ($P>0.05$). At 21d, no significant differences between treatments were observed in *C. jejuni* colonization. Both at 35 and 42d, MGOA at high doses (2 and 3%) reduced *C. jejuni* counts compared to the other treatments (6.45, 5.68, 3.78 and 3.67 log₁₀ CFU/g at 35d, $P = 0.009$ and 6.44, 5.74, 3.98 and 4.16 log₁₀ CFU/g at 42d, $P=0.049$; for C, C+MGOA(1%)+P, C+MGOA(2%)+P and C+MGOA(3%) + P, respectively). Differences came from the proportion of non-infected birds (12.5, 25.0, 62.5 and 68.8% non-infected birds at 35d, $P = 0.002$, and 7.1, 25.0, 62.5 and 56.3% non-infected birds at 42d, $P = 0.005$, for C, C+MGOA(1%)+P, C+MGOA(2%)+P and C+MGOA(3%)+P, respectively). It is concluded that the combination of MGOA and P has a significant effect in reducing *C. jejuni* population at cecum level, but high levels of MGOA are necessary to show such reduction.

Keywords: mono-glycerides, organic acids, probiotics, *C. jejuni*, broiler

S10-0057 Effect of supplementing a combination of organic acids and mono-glycerides and a probiotic during different times of administration on *Campylobacter* colonization in broilers

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An experiment was conducted within the EU-FP7 project CAMPYBRO in order to evaluate the appropriate time of administration of a combination of a blend of mono-glycerides and organic acids (MGOA) and a probiotic (*B. subtilis* C-3102, P) on *C. jejuni* counts in broilers. There were four treatments applied from 1-42d of age: T1, Control diet (C), T2, C+MGOA (0.5% from 1-10d, 2.0% from 10-21d)+P (0.01% from 1-21d), T3, C+MGOA (2.0% from 21-42d)+P (0.01% from 21-42d) and T4, C+MGOA (0.5% from 1-10d, 2.0% thereafter)+P (0.01% from 1-42d). At 14d, all broilers (232 one-d-old Ross 308 broilers, male&female, 50%) were orally gavaged with 100 µl of a solution containing 1x10⁵ CFU/ml of ST-45 and ST-21 *C. jejuni* strains. At 21, 35 and 42d, ceca from 16 birds per treatment were collected and *C. jejuni* counts determined (ISO 10272). Data expressed as log₁₀ CFU/g ceca content were first tested for normality and then analysed by the nonparametric test of Kruskal-Wallis (SPSS v.19.0). No significant differences between treatments were observed in performance for any of the periods studied ($P>0.05$). At 21d, broilers receiving MGOA+P (T2 and T4) showed less *C. jejuni* counts than non-supplemented birds (T1 and T3) (7.85, 6.80, 7.21, and 6.60 log₁₀ CFU/g for T1 to T4, respectively, $P=0.026$). The observed mean reduction in *C. jejuni* came from a log reduction rather than from an effect on the number of non-infected broilers. The effect was lost thereafter and no significant differences between treatments were observed in *C. jejuni* colonization at 35 and 42d (7.18, 7.48, 7.53 and 6.88 log₁₀ CFU/g at 35d, $P = 0.930$ and 7.05, 6.69, 6.10 and 6.28 log₁₀ CFU/g at 42d, $P = 0.228$ for T1 to T4, respectively). It is concluded that the combination of MGOA and P have a significant effect in reducing *C. jejuni* population at 21d, but the positive effect was lost thereafter.

Keywords: mono-glycerides, organic acids, probiotics, *C. jejuni*, broiler

S10-0058 Interaction of a combination of organic acids and mono-glycerides and a probiotic with a functional diet on *Campylobacter* colonization in broilers

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An experiment was conducted within the EU-FP7 project CAMPYBRO in order to evaluate the effect of a combination of a blend of mono-glycerides and organic acids (MGOA) with a probiotic (*B. subtilis* C-3102, P) added to a control (C) or a functional (F) diet on *C. jejuni* counts in broilers. There were four treatments factorially arranged applied from 1-42d of age, based on two types of diet: C and F with or without MGOA (1.5% from 1-10d, 2.5% thereafter) + P (0.01%). The F diet included 5% oat hulls and 7.5, 12 and 15% whole wheat from 1-10, 10-21 and 21-42d, respectively. At 14d, all broilers (216 one-d-old Ross 308 broilers, male&female, 50%) were orally gavaged with 100 µl of a solution containing 1x10⁵ CFU/ml of ST-45 and ST-21 *C. jejuni* strains. At 21, 35 and 42d, ceca from 14 birds per treatment were collected and *C. jejuni* counts determined (ISO 10272). Data expressed as log₁₀CFU/g ceca content were first tested for normality and then analysed by the nonparametric test of Kruskal-Wallis (SPSS v.19.0). Birds fed the C diet were heavier than birds fed the F diet at 10d (226, 217, 203 and 210 g, for C, C+MGOA+P, F and F+MGOA+P, respectively, P=0.003), but differences disappeared thereafter. No other significant differences between treatments were observed in performance for any of the periods studied (P>0.05). Supplementation with MGOA+P reduced *C. jejuni* colonization both in C and F diets at 35 d (6.57, 3.70, 6.46 and 4.91 log₁₀CFU/g for C, C+MGOA+P, F and F+MGOA+P, respectively, P=0.036). This reduction occurred mainly due to the greater number of non-infected birds in MGOA+P treatments (14.3, 71.4, 7.1 and 35.7% non-infected birds for C, C+MGOA+P, F and F+MGOA+P, respectively, P=0.001). However, the positive effect of MGOA+P on *C. jejuni* reduction was lost at 42d (P>0.05). It is concluded that the combination of MGOA and P have a significant effect in reducing *C. jejuni* population at cecum level at 35d. However, for unknown reasons, this effect was lost at 42d.

Keywords: mono-glycerides, organic acids, probiotics, *C. jejuni*, broiler

S10-0059 Interaction of a combination of organic acids and mono-glycerides and a probiotic on *Campylobacter* colonization in broilers

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An experiment was conducted within the EU-FP7 project CAMPYBRO in order to evaluate the interaction of supplementing a combination of a blend of mono-glycerides and organic acids (MGOA) and a probiotic (*B. subtilis* C-3102, P) alone, or in combination, on *C. jejuni* counts in broilers. There were four treatments applied from 1-42d of age: Control diet (C), C+MGOA (0.5% from 1-10d, 2.5% thereafter), C+P (0.01%) and C+MGOA+P. At 14d, all broilers (240 one-d-old Ross 308 broilers, male&female, 50%) were orally gavaged with 100 µl of a solution containing 1x10⁵ CFU/ml of ST-45 and ST-21 *C. jejuni* strains. At 21, 35 and 42d, ceca from 16 birds/treatment were collected and *C. jejuni* counts determined (ISO 10272). Data expressed as log₁₀CFU/g ceca content were first tested for normality and then analysed by ANOVA or the non-parametric test of Kruskal-Wallis (SPSS v.19.0). Birds fed the C diet were heavier than birds receiving MGOA at 21d (764a, 720bc, 759ab and 699c g, for C, C+MGOA, C+P and C+MGOA+P, respectively, P=0.003), but differences disappeared thereafter. No other significant differences between treatments were observed in performance for any of the periods studied (P>0.05). Supplementation with MGOA alone, or in combination with P, reduced *C. jejuni* colonization at 21 d (P<0.001). At 35d, MGOA+P reduced *C. jejuni* counts compared to the other treatments (6.82, 6.56, 6.39, 4.45 log₁₀CFU/g for C, C+MGOA, C+P and C+MGOA+P, respectively, P=0.024). Differences came from the proportion of non-infected birds (6.3, 6.3, 12.5 and 43.8% non-infected birds for C, C+MGOA, C+P and C+MGOA+P, respectively, P=0.012). The positive effect of the combination of MGOA and P on *C. jejuni* reduction was also observed at 42d (8.51a, 7.30bc, 8.28ab and 6.89c log₁₀CFU/g for C, C+MGOA, C+P and C+MGOA+P, respectively, P=0.001). It is concluded that there is a synergism between MGOA and P and the combination of MGOA and P have a significant effect in reducing *C. jejuni* population at cecum level.

Keywords: mono-glycerides, organic acids, probiotics, *C. jejuni*, broiler

S10-0060 Real Time-Loop Mediated Isothermal Amplification (RT-qLAMP) targeting S1 gene for rapid detection of Avian Reovirus in clinical/meat samples

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Chicken meat has increased its proportion of the total meat market because of price advantage, easy availability, palatability and a positive health image among consumers. People demand in the area of food safety appears to be for a product without chemical toxicants and without disease causing pathogens. Today contaminations with pathogenic microorganism become a major issue for the chicken industry. Avian reovirus (ARV) causes several severe conditions, including viral arthritis, malabsorption and pale bird syndrome which cause significant loss in World's poultry industry. The aim of this study is to design a rapid and sensitive Real Time-Loop Mediated Isothermal Amplification test (RT-qLAMP) and to use this developed test for detection of ARV in clinical/meat samples. For creation of probe, we annealed oligonucleotide sequence Fd complementary to flap region (F1c) with a Cy5 fluorophore on 3' end spectrally overlapping with the Forward Internal Primer tagged with Iowa Black quencher on 5' end. This duplex primer retains its function as a LAMP primer, but during synthesis from the reverse direction, the flap duplex is separated which results in detection of amplification by release of quenching. Avian reovirus S1 gene encoding for sigma C protein is chosen as target gene for development of RT-qLAMP. The developed test specifically detects presence of ARV but not the other poultry viruses i.e. New Castle Disease virus (NDV), and Infectious Bursal disease (IBD) in the samples. The designed test is more sensitive than Real Time PCR and is free from false positive result which is a limitation of LAMP PCR. 50 suspected clinical and artificially spiked samples were analysed using this test, out of which 5 were found positive for ARV. The developed test is a rapid sensitive test having analytical sensitivity of 1 fg RNA and can be used for mass screening of ARV in clinical/meat sample.

Keywords: Avian Reovirus, LAMP, diagnosis

S10-0061 Effects of the raising model on physical properties of muscle in Chengkou mountain chicken

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Chengkou Mountain chicken is an excellent local breed in China, and most of chicken were raised in the free-range model. However, the free-range model of raising native chicken in China is gradually transforming into barn feeding. This experiment was conducted to study the effects of raising model on physical properties of muscle in Chengkou mountain chicken. Six hundreds female Chengkou mountain chicken (at 1 d of age) were randomly allocated into 2 groups (with a total of 6 replicates per group and 50 chickens per replicate), which were control group and experimental group respectively. All chickens were offered the same conditions from 1 to 60 days, the control chickens were raised in the free-range raising model and experimental group in barn feeding from 60 to 120 days. At 120 d of age, 6 chickens from each per replicate were randomly selected and processed for determination of physical properties of breast meat. Data were analyzed using t-test of SPSS 17. The results showed that raising Chengkou Mountain chicken in barn feeding increased fresh meat shear force (29.6956 and 24.4537 N, respectively; $P = 0.0013$), and pH (6.1875 and 6.0663, respectively; $P = 0.0052$). Raising model did not affect cooked meat shear force (42.9742 and 40.1475 N, respectively; $P = 0.4162$), light flesh color (88.3303 and 88.0923, respectively; $P = 0.5931$), and drip loss (8.54 and 7.94 %, respectively; $P = 0.4329$), cooking loss (29.68 and 28.38 %, respectively; $P = 0.3175$), and cooked meat percentage (0.7031 and 0.7161 %, respectively; $P = 0.3175$) in breast muscles of chicken raised in barn feeding and in the free-range. In conclusion, it suggests that raising Chengkou Mountain chickens in barn feeding may reduce muscle tenderness and increased pH, but had little effects on other physical properties of breast muscle. Chengkou Mountain chickens in barn feeding, in comparison to free-range, might be feasible and effective in the production of chicken.

Keywords: physical properties, chicken, muscle, raising model

S10-0062 Tissue residue distribution and residue elimination of dietary Phenylethanolamine A in broiler chickens

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Although Phenylethanolamine A (PEA, a new β -adrenal agonist) was prohibited to use in the feed and drinking water for livestock in China since 2010, we had detected it in animal products. The experiment was conducted to study the tissue distribution and residue elimination of PEA. A total of 400 21-day-old broiler chickens (AA) with similar initial body weight (0.812 ± 0.006 kg) were selected and randomly allotted to 4 treatments with 10 replications of 10 birds. There were two phases, PEA treatment phase (21 to 42 d) and depletion phase (42 to 56 d). During the treatment phase, the PEA was added at levels of 0, 10, 20 and 40 mg/kg in a corn-soybean meal basal diet. At 42 d, one chicken that was close to average weight from each cage was selected to take blood, tissues including liver, lung, kidney, and pectoral major. For the depletion phase, all the treatments were changed to basal diet. Blood and tissues were obtained as the above method at 1, 2, 4, 7 and 14 d, to determine the residue depletion of PEA. The results showed that: 1) PEA were observed in blood and tissues (liver, kidney, lung and pectoral major), with lung ($57.73-353.03$ ng/g, $P < 0.01$) the largest residual content, followed by liver ($8.10-19.26$ ng/g, $P < 0.01$), kidney ($4.02-10.73$ ng/g, $P < 0.01$), pectoral major ($2.10-7.54$ ng/g, $P < 0.01$) and blood ($0.658-4.60$ ng/g, $P < 0.01$), ($P < 0.01$) with the 10-40 mg/kg PEA in feed. 2) The residue of PEA in blood and tissues was eliminated slowly. Except lung, there was none of PEA residue in blood and tissues 14 days after switched to basal diet. The elimination of PEA in blood and tissues was conformed to exponential regression model. Conclusions: 1) The residue of PEA was highest in lung than other tissues. 2) The elimination way of PEA was conformed to exponential regression and was the same when supplied different levels of PEA. **Keywords:** Phenylethanolamine A(PEA), broiler, tissue deposition, residue depletion

S10-0063 Influence of gradual changes of carbon dioxide and oxygen concentrations during controlled atmosphere stunning on broiler behaviour

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Controlled Atmosphere Stunning (CAS) is usually applied in two phases: induction phase (max 40% CO₂) and completing phase (higher CO₂ concentration after loss of consciousness). Adding oxygen to the induction atmosphere is beneficial for a smooth induction of unconsciousness. Where originally one minute induction phase, followed by two minutes completing phase were used, lately a longer stunning time with a more gradual increase of CO₂ is often applied. While a slow initial increase of CO₂ concentration can lead to a longer time until birds are effectively rendered unconscious, a more gradual transition after the loss of consciousness, from the induction atmosphere to the completing atmosphere, may have beneficial effects with regard to reducing convulsions, therefore potentially reducing carcass damage. However, quantitative results with different steps of gradual changes of CO₂ and O₂ from induction to completing atmosphere are not yet available. In the present study, birds were therefore subjected to different settings of stepwise CO₂ increase and O₂ decrease and different transition times. The study was carried out under practical conditions in a commercial CAS tunnel, consisting of 5 separate sections of one minute dwell time, each with a separate gas control unit. Actual gas concentrations were continuously recorded. Birds of different flocks were allocated to two weight classes (heavy: 2,7kg, light: 1,9kg). Video recordings were obtained from each of the 5 sections for 15 minutes per flock (each video representing 3.300 birds) and number of birds showing signs of convulsions and wing flapping were assessed. Results showed that birds had reliably lost posture before transfer to concentrations above 40% CO₂. Shorter dwell times in the transition from induction to completing phase did not increase the occurrence of convulsions compared to the standard setup, while a lower initial O₂ concentration increased number of convulsions and wing flapping.

Keywords: CAS, broiler chickens, oxygen, carbon dioxide, behaviour

S10-0064 The occurrence of wooden breast in a Danish flock of broiler chickens

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The aim of this study was to investigate the frequency of wooden breast (WB) and to detect the potential association with age, growth and selected welfare parameters. From a single flock we sampled 198 birds and 200 birds at the age of 20-21 days and 30-31 days, respectively. The following parameters were recorded for all birds: Carcass weight, weight of the breast filets, WB, footpad dermatitis and wing motility. The weight of the heart and liver was recorded in every fifth of the WB birds and in all of the WB-positive birds. For 165 birds (30-31 days) the gender was recorded. Statistical analysis was performed using the Fisher's exact and logistic regression to analyse the relationship between two categorical variables and dichotomous outcome with one or more independent variables. The frequency of WB was significantly higher in birds at 30-31 days (36 (18%)) compared to birds at 20-21 days (nine (5%)), $P < 0.000$. The mean carcass weight for the birds was 885g and 1781g at 20-21 and 30-31 days, respectively. There was a significant difference between carcass weight of birds without WB=1762g and WB-positive birds=1869g ($p < 0.01$). The mean breast filet weight of birds without WB=269g, and mean breast filet weight of WB-positive birds=305g ($P < 0.000$). The breast ratio (breast weight/carcass weight) for birds without WB=0.15, and 0.16 for birds with WB ($P < 0.000$). There was no effect of gender or weight of liver or heart, when the carcass weight were included in the model. There was no effect of footpad dermatitis on the frequency of WB. However, reduced wing motility was highly associated with the presence of WB ($P < 0.000$) for the birds at 30-31 days. The frequency of WB was associated to the carcass weight and the breast weight. The breast ratio was highly associated with WB. We cannot determine whether high weight and breast ratio causes WB or if WB causes swelling of the breast, and thereby increasing the breast ratio.

Keywords: wooden breast, meat quality, quality defect, muscle anomaly

S10-0065 Impact of wooden breast, pH and water-holding capacity and consequences for microbial composition

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Impact of wooden breast; pH and water-holding capacity and consequences for Wooden breast (WB) is a phenomenon where pectoralis major, is characterised by pale and bulging hard areas resulting in several implications for the quality of the fresh and processed products. The prevalence of this quality defect has dramatically increased within the past 5-10 years, and severely affected breast meat is rejected or degraded, resulting in considerable economic losses to the poultry industry. Water availability and pH are two very important parameters when investigating conditions of importance for microbiological growth and hence shelf life of the product. The objective of this study was to investigate the impact of WB on the pH and water-holding capacity (WHC) and its consequences for the microbiological quality. Large breast filets were sampled from a commercial Danish slaughter plant from the slaughter line downstream of veterinary control and categorized as control (W0B), slight WB (WB1) or severe WB (WB2). The day of slaughter (day 0), the day after (day 1) and three days after slaughter (day3) samples were analyzed for intramuscular and surface pH and WHC as drip loss and the relative amount of mobile water (T22 area) by NMR as well as cooking loss. The pH was higher (intramuscular and surface), drip loss and cooking loss increased and the mobile water pool larger in WB samples compared to the control samples. A quantitative but also qualitative determination of the dominating microorganisms by 16S rRNA sequencing at the end of shelf-life has shown, that these effects have an impact on the composition of the shelf-life limiting microflora.

Keywords: wooden breast, meat quality, water-holding capacity, microbiology

S10-0066 The relationship between calpain and drip loss of chicken breast during ageing

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As the main proteases in all cells, the calpain system plays a major role in myofibrillar degradation. So CAPN1, encodes the large subunit of μ -calpain, is used for improve meat quality of chicken. In the former study, we found SNPs in CAPN1 gene associated with drip loss of chicken breast muscle and the muscle fiber density and diameter. To learn the influence of CAPN1 on the meat quality of chicken breast during the postmortem, we detected calpain activity and multiple index of meat quality during ageing. In this study, thirty males from a cross breed were sacrificed and stripped the breast for ageing in 4° C. Drip loss, pH, calcium concentration of cytoplasm and muscle fiber characteristic were measured at 0h, 3h, 6h, 12h, 18h and 24h during ageing. And determined the calpain activity and the desmin expression at different ageing time. The results showed that during 0h to 12h, pH was gradually decreased, calcium concentration was gradually increased, and the calpain activity was increased. And during this time, the degradation of desmin was slow and the muscle fiber contracted intensely. While during 12h to 24h, pH was gradually increased, calcium concentration was rapidly decreased, and the calpain activity was rapidly decreased. And during this time, the degradation of desmin was fast and the muscle fiber gradually dilated. According to the results, we predicted that CAPN1 may influence drip loss of chicken breast by degrades the desmin during ageing and it may affect the muscle fiber degradation. As the specific cytoskeletal protein in muscle cells, desmin degradation could speed up the muscle fibers relaxation, and this could increase the space to storage water and the net charge to attract water. So this study can provide an evidence for the molecular mechanism of CAPN1 effect on the drip loss of chicken breast.

Keywords: CAPN1, drip loss, desmin, muscle fiber, chicken

S10-0067 The residual and elimination of mineral oils of vaccine's adjuvant in chicken eggs and edible tissue

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Mineral oils of vaccine's adjuvant incorporated into vaccines to enhance immunogenicity. Mineral oils were comprised of highly complex mixtures that including principally straight and branched alkanes and polycyclic aromatic hydrocarbons, are potential violate residues in food animal-derived products. To investigate the disposition and elimination of n-alkanes and PAHs, 120 1-day-old Jingfen layer chickens injected oil vaccines as the routine immune program were used. The trial period last for 430 days. PAHs and n-alkanes in egg and edible tissue samples were analyzed using a gas chromatography/mass spectrometry (GC-MS). 3 eggs were collected randomly from every repeat at 159d, 166d, 180d, 201d, 235d, 271d. 6 chicken were chosen randomly from every repeat to slaughter at 280d, 330d, 380d, 430d, collecting edible tissues. The results showed that PAHs and n-alkanes transferred to eggs is too little, which has no effect on the egg quality. The residue time of the PAHs and n-alkanes in the chicken edible tissue is long. It need about 210d withdrawal to reach the international food security standard. The alkanes with smaller molecular weight of mineral oil, such as n-hexadecane, n-heptadecane, n-octadecane, are easy to metabolize and transfer to other part of chicken body. The residue of the mineral oil in injection site is most and it can transfer to other tissues and organs after it was inject to the chicken. The residual amount is larger and the eliminate rate is lower in liver and kidney than other tissues. This study was financially supported by Yang-zhou prospective study of agriculture fund (yz2014145).

Keywords: mineral oil, layer chicken, polycyclic aromatic hydrocarbons, residual

S10-0068 Effect of Moringa Oleifera leaf powder supplementation on morphometric characteristics of pectoralis major muscle of broiler chicken

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This experiment was conducted to determine the effect of dietary supplementation of Moringa oleifera leaf powder (MOLP) on morphometric characteristics and selected meat quality parameters of breast muscle in broilers. Rich nutrient profile, multiple medicinal uses and antioxidant properties of M. oleifera lead to a possibility of utilizing its leaf powder as an efficient and cost-effective feed additive for broilers. Day-old broiler chicks (n=100) were randomly divided into five groups with four replicates each (n=5). Birds were fed a corn-based basal diet or the same diet supplemented with 0.6, 0.9, 1.2 and 1.5% MOLP with an ad libitum access to fresh water. Trial was conducted for 35 days, after which two birds per replicate were de-capitated to collect breast muscle (pectoralis major) for determination of histo-morphometric indices, water holding capacity, and pH at 0, 12, 24 hours. Data were analysed through one way analysis of variance and Duncan's multiple range test. All differences were considered significant at $p < 0.05$. MOLP supplementation had no significant effect on muscle fibre density but muscle fibre diameter was significantly higher ($p < 0.05$) in 1.2% and 1.5% MOLP supplemented groups compared to the control group. The water holding capacity of 0.6%, 0.9% and 1.5% MOLP supplemented groups was significantly higher ($p < 0.05$) than the control group. The pH of MOLP supplemented groups showed higher values ($p < 0.05$) at all three intervals when compared to control group. It may be concluded that breast muscle (pectoralis major) of broilers supplemented MOLP, particularly at inclusion level of 1.5%, showed better results for parameters important for shelf life as well as consumers' preference.

Keywords: phytobiotics, breast muscle, skeletal muscle, water holding capacity, meat pH

S10-0069 Monitoring Salmonella in broiler meat produced by large-scale poultry processing plants in Sri Lanka

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The objective of this study was monitoring the occurrence of Salmonella in broiler meat produced in large-scale, quality certified poultry processing plants in Sri Lanka. Poultry meat is one of the most popular meat types in the country and a greater proportion of commercial broiler meat is produced by large-scale processing plants. Verification of Salmonella controls on broiler chickens at the end of primary processing is a requirement for certified poultry processing companies. According to Sri Lanka standard for poultry meat (SLS 1161:2003) there shouldn't be Salmonella in 25g of meat. Over a period of 20 months (February 2014 to October 2015) a total of 50 samples from large-scale processors, received by the Food Microbiology laboratory in the Faculty of Veterinary Medicine and Animal Science were included in the monitoring program. Randomly selected meat samples sent under refrigerated condition by processors were tested for Salmonella according to the method described in ISO 6579 standard. Thirty five samples out of 50 were from two processing plants. Hence, data generated from the samples sent by the two processors were analyzed as the preliminary results of the monitoring program and presented here. In the year 2014, five samples were positive for Salmonella out of 13 test samples accounting 33% contamination. In the year 2015 only two samples were positive out of 22 samples indicating lower level of contamination (9%) compared to previous year. The percentage of reduction in Salmonella contaminated meat was due to corrective actions taken along the processing line. Therefore, monitoring by an independent laboratory and sharing data with the processors was effective on improving the product quality. However, identification of Salmonella contamination in meat produced by processing plants, even with food safety management systems in place, was alarming. The findings indicates the need of much more stringent control of the pathogen from farm to fork.

Keywords: Salmonella, poultry processing

S10-0070 Carcass and Meat Assessment of broiler chickens fed raw benne seeds (*Sesamum indicum*) basal diets with or without lysine supplementation

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This study was carried out to assess the effect of raw benne seeds (RBS) (*Sesamum indicum*) basal diets with or without lysine supplementation on carcass and meat characteristics of broiler chickens in an S8-week feeding trial. Raw benne seeds was incorporated into the diets at 15% and 30% while lysine was incorporated at 0.25% and 0.5% thus 7 diets were formulated as: T0 = Control; (No raw benne seeds and lysine); T1 = 15% RBS + 0% lysine, T2 = 15% RBS + 0.25% lysine; T3 = 15% RBS = 0.5% lysine; T4 = 30% RBS + 0% lysine, T5 = 30% RBS + 0.25% lysine; T6 = 30% RBS + 0.5% lysine. 140 unsexed broiler chicks of Marshall strain were randomly allotted into 7 dietary groups, each group replicated 4 times at 5 birds per replicate. Fasted live, dressed carcass, and primal cuts weights as well as their percentages with exception of the back at T6 all reduced significantly ($P < 0.05$) in carcasses of birds fed 30% RBS than those fed 15% RBS with lysine and without lysine supplementation up to 0.5%. There were significant ($P < 0.05$) increase in cooking loss, thermal shortening and shear force values, while water holding capacity and cooking yield decreased ($P < 0.05$) in meat of birds fed 30% RBS with or without lysine. Sensory characteristics were significantly ($P < 0.05$) higher in meat of birds fed 15% RBS with or without lysine supplementation than birds fed 30% RBS. Overall acceptability was higher ($P < 0.05$) in meat from birds fed 15% RBS with T2 having the highest ($P < 0.05$) acceptability score. The best significant improvements in the carcass and meat was in birds fed 15% RBS supplemented with 0.5% lysine, while meat acceptability was higher in those fed 15% RBS with 0.25% lysine supplementation.

Keywords: assessment, benne seeds, broiler chicken, carcass and meat, lysine supplementation

S10-0071 Influence of food handlers' compliance with procedures of poultry carcasses contamination: a case study concerning evisceration in broiler slaughterhouses

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Our study aimed at investigating whether compliance of food handlers with procedures on setting and controlling evisceration process parameters could explain differences in microbial concentrations on carcasses between slaughterhouses. The study was conducted in two commercial broiler chicken slaughterhouses. Analysis of documentation provided insight in the adequacy of procedures, and observational studies revealed insight in compliance with procedures by using a set of criteria for evisceration control. The frequency of carcasses with visible faecal contamination was counted and *E. coli* concentrations on carcasses classified based on visible contamination was analysed. Food handlers' knowledge, attitude and practices related to evisceration control tasks were analysed based on a validated questionnaire. Documentation analysis revealed obvious differences in the procedures between slaughterhouses. In the slaughterhouse with advanced procedures, the food handlers more often complied with these procedures and a lower frequency of carcasses with visible faecal contamination was observed. Carcasses contaminated with visible faecal spots, even at a low level, carried significantly higher concentrations of *E. coli* than visibly clean carcasses. Food handlers in both slaughterhouses revealed a good knowledge level, however their attitude differed. In one slaughterhouse, where food handlers complied more frequently with procedures their attitude was at a good level, and practices at good and moderate levels. In the other slaughterhouse the attitude of food handlers was at moderate level and practices at moderate and poor levels. In conclusion, this study suggest that management factors like availability of adequate monitoring procedures and food handlers' compliance with these procedures may influence the bacterial concentrations on carcasses. To validate these findings an intervention study is needed.

Keywords: processing hygiene, food handler, compliance with procedures

S11- 0001 Influence of temperature on the organ development in the chicken

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The chicken embryo cannot regulate its own temperature; consequently, it is important to incubate eggs at a temperature that optimizes hatchability, defined as 37.5 to 37.8°C. Large deviations from this have been known to interfere with development, hatchability and post hatch survival and performance. One thousand and ten pathogen free eggs were divided into three groups. One group was incubated as control (incubation temperature - 37.8°C; relative humidity - 50-55% for 18 days and 60-65% for 3 days), and the other two groups challenged with: A) constant high incubation temperature (38.9°C); B) constant low incubation temperature (36.7°C). Eggs were removed from the incubator and sequential break-outs performed to evaluate the effects of the different manipulations on embryonic development. Microscopic evaluation was thoroughly performed for all embryos. However, changes were only found in the gizzard and bursa of Fabricius. Embryos exposed to Treatment A exhibited vacuolization of the glandular epithelium of the gizzard mucosa from day 12 onward. Cellular overdistension with content and cell rupture were frequent, with formation of irregularly-shaped cavities in the mucosa. The mucosa in these embryos appeared disorganized, irregular and less cellular, when compared to control and Treatment B embryos. In the bursa, a greater number of lymphoid follicles was at first observed in Treatment A embryos (days 12-14). By day 18, follicles appeared in similar numbers and dimensions to controls but contained scarcer numbers of lymphoid cells. In Treatment B embryos, the bursa of Fabricius consistently appeared to have a lower number of follicles (which were also smaller in size) and a diminished number of lymphoid cells when compared to controls. These findings suggest that increase in temperature has a detrimental effect on the development of the gizzard and the bursa, which should be taken into consideration due to relevant consequences that may arise after hatching.

Keywords: chicken embryo, temperature, incubation, Gizzard, Bursa of Fabricius

S11- 0002 Effect of prolonged storage of duck eggs with and without periodic warming on hatchability and growth and carcass quality of hatched ducklings

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In pedigree breeding it would be necessary to prolong the storage time for hatching eggs to have more uniform offspring. Sometimes it is necessary to store hatching eggs up to 3 or 4 weeks. In 2 experiments it was compared the effect of storage duck eggs with periodical warming up to 37.4°C for 3 hours at the 3rd day and for 2 hours at days 8, 11, 14, 19, 21, 23, 25 and 27 with storage duck eggs without warming procedure on hatching rate. The negative effect of storage duration on hatchability was more pronounced in the group without periodic warming. In exp. 1 hatchability was 89.1 % , 87.0 % , 80.8 % and 71.1 % with warming and 85.7 % , 86.4 % , 70.0 % and 64.2 % without warming for storage duration of 1, 2, 3 and 4 weeks. In exp. 2 the results were: 83.4 % , 81.0 % , 77.7 % and 70.9 % with warming procedure and 79.9 % , 80.2 % , 77.8 % and 61.4 % without warming procedure, respectively. The reason for decreased hatchability was increasing of early embryonic mortality, of dead in shell and of delayed hatching, but pronounced in the group without warming. The hatched ducklings (1644 in exp. 1; 360 in exp. 2) were tested in single boxes (3. - 6. week) their growth, carcass quality and feed intake. All 6 weeks old ducks were slaughtered. The storage of hatching eggs with warming resulted higher 2-week bodyweight than of hatching eggs without periodic warming (585 to 545 g exp. 1; 603 to 572 g exp. 2). Also the 6-week bodyweight was higher in exp. 2 in the group with warming procedure (3173 g to 2928 g), but not in exp. 1 (3102 g to 3094 g). Feed efficiency was improved in the groups with periodic warming in both experiments significantly (exp. 1: 442 to 437 g; exp. 2: 403 to 377 g). The breast file percentage was higher by periodic warming in experiment 1, but not in exp. 2. (exp.1: 21.8 % to 21.4 % , exp. 2: 22.6 % to 22.7 %). There was no significant effect of duration of storage. Also interactions between duration of storage and storage conditions were unimportant.

Keywords: storage duration, periodic warming, hatching rate, bodyweight, feed efficiency, breast file

S11- 0003 The influence of position of duck eggs within a tray on the efficiency of incubation

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Incubation of eggs of waterfowl has its own specifics due to the certain biological peculiarities of both egg structure and embryonic development in these eggs. The efficiency of incubation could be improved by different methods. It is known that the position of eggs within egg-trays can influence growth and development of allantois. Lower pressure in the closure zone of allantois is known to improve its development and hatchability of eggs; e.g. it occurs when eggs within a tray are oriented horizontally. Eggs from ducks of the same age were allotted in 2 groups. In control group 1 eggs were oriented in trays vertically, in experimental group 2 horizontally during the entire incubation. The incubation regime was similar for both groups. Totally 2500 eggs were incubated, 40 eggs were broken for analysis. 10 embryos and 20 day-old ducklings from each group were slaughtered for analysis. The closure of allantois and utilization of protein occurred earlier when eggs were incubated in horizontal position. After 21 days of incubation in experimental group there was no residue of egg white while in control avg. 2.1% of egg white remained unused by developing embryos. The hatch of ducklings in experimental group was concurrent and finished 4 hrs earlier than in control group. Hatchability of eggs in group 2 was 8.4% higher compared to control ($P<0.02$). The improvements in embryonic deaths in group 2 (compared to control) were 2.0% during 1st week of incubation; 4.0% during 2nd week and 3.7% during pre-hatch period. All ducklings in group 2 were A-grade while in control 1.0% of ducklings were culled. More comfortable conditions of incubation favorable for embryonic development can therefore improve hatchability of eggs and quality of hatched ducklings.

Keywords: duck eggs, hatchability, egg position within a tray

S11-0004 Two methods that prevent hatchability decline due to 21 days of egg storage

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Egg storage beyond 7 days decreases hatchability. Short Incubation Periods During Egg Storage (SPIDES) or a slow warming profile at the start of incubation can reduce negative effects of egg storage on hatchability. In the current study, the effect of only SPIDES or a combination of SPIDES and a slow warming profile on hatchability was investigated when Ross 308 eggs from a 43 weeks old breeder flock were stored for 3 (short storage) or 21 days (long storage; $n=1350$). SPIDES was performed at D7 (1xSPIDES) or at D7 and 14 (2xSPIDES) of storage. During SPIDES, eggs were warmed to an eggshell temperature (EST) of 37.8°C using a linear warming curve of 10 hours. When an EST of 37.8°C was reached, eggs were cooled inside the incubator to an EST of 25°C. Afterwards eggs were returned to the storage room where air temperature was maintained at 18°C. At the start of the incubation process, EST was increased linearly from 21°C to 29.4°C in 5 hours and from 29.4°C to 37.8°C in 5 hours (WP5-5) or in 17 hours (WP5-17), resulting in 1 short storage treatment (WP5-5 short storage) and 3 long storage treatments: WP5-5 long storage, WP5-5 2xSPIDES, and WP5-17 1xSPIDES. Data were analyzed as a complete randomized design. Hatchability of set eggs of WP5-5 short storage was 25.8% higher than of WP5-5 long storage (89.6% vs. 63.8%). In comparison to WP5-5 long storage, WP5-5 2xSPIDES improved hatchability by 21.4% and WP5-17 1xSPIDES improved hatchability by 20.7%. Hatchability of WP5-5 2xSPIDES and WP5-17 1xSPIDES did not differ from each other and did not differ from WP5-5 short storage (85.2% and 84.4% vs. 89.6%, respectively; $P<0.001$). It can be concluded that WP5-5 2xSPIDES and WP5-17 1xSPIDES prevented any decline in hatchability during 21 days of storage in the current study.

Keywords: SPIDES, slow warming profile, hatchability, storage

S11-0005 The influence of duration of egg storage on the quality of turkey poults

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Quality and livability of poults depend on many factors, the most influential being quality of incubation eggs and duration and conditions of egg storage. In practice eggs are often stored to accumulate an amount sufficient for incubators hence it's important to be aware of the influence of the duration of egg storage prior to incubation on the quality of hatched poults. In a trial turkey eggs were allotted in 4 analogous groups (by egg weight), 1 control and 2-4 experimental. Eggs in experimental groups 2-4 were stored prior to incubation at 14-16°C during 6; 9 and 12 days, respectively; in control group 1 eggs were stored for 3 days. Hatched poults (30 birds per group) were raised to 14 days of age in Institute's vivarium. The percentage of off-grade poults in experimental groups was higher by 0.7-1.8% compared to control. In group 4 relative weight of liver in day-old poults (related to live bodyweight) was higher by 0.19% ($P<0.02$) compared to control. Concentrations of vitamin A and carotenoids in liver in experimental groups 2-4 were lower compared to control by 8.58-11.4; 7.6-11.1 and 11.2-19.1 µg/g, respectively. Pre-incubatory egg storage was found to influence the post-hatch growth in turkey poults. At 7 days of age live bodyweight in experimental groups was significantly lower compared to control (by 4.2-7.4 g, $P<0.01$, $P<0.02$). At 14 days of age this difference grew 3-4 times higher: live bodyweight in experimental groups 2-4 was lower compared to control by 16.5 g ($P<0.02$), 16.6 g ($P<0.05$) and 27.1 g ($P<0.001$). The longer was the duration of egg storage the lower were live bodyweight and daily weight gains in hatched poults.

Keywords: turkey eggs, duration of egg storage, quality of turkey poults

S11-0006 Hypoxia related gene expression in embryonic liver, lungs, brain and muscle during the incubation of Nigerian Indigenous Chicken eggs

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Hypoxia is a common fetal stressor that can induce profound abnormalities in the development of the cardiovascular and other systems during early development of the chickens embryo owing to restricted oxygen delivery to somatic tissues. Consequently, it impacts on hatchability. This study investigated the expression of hypoxia-inducible Factor-1α (HIF-1α) mRNA in the embryonic liver, lungs, brain and muscle of Nigerian Indigenous Chicken (NIC) at day 15, 18 and 20 of incubation. Seven hundred fertile hatching eggs of the breed were obtained and set for incubation in a single stage incubator at the hatchery of the Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria. During the incubation period, ten eggs were randomly selected from the incubator at day 15, 18 and 20 of incubation. Eggs were weighed and broken and the embryo was aseptically removed for dissection. Experimental samples (which included the Lung, Liver, whole brain and muscle) were collected into eppendorf tubes and stored at -80°C until the extraction of total RNA and RT-PCR analysis. The amplified products were viewed on a 1% agarose gel. The bands indicating the mRNA expression of the genes were semi-quantitatively analyzed by densitometry. Data generated were subjected to one-way analysis of variance, with age as the dependent variable. The level of expression of HIF-1α was observed to be greater in the liver than in the lungs of Normal Indigenous Chicken breeds. HIF-1α level in the liver increased with age of the embryo but decreased with age in lungs. The HIF-1α mRNA expression increased significantly in the liver from day 15 to day 20 and also in the brain from day 15 to day 20. It was concluded that the probability of susceptibility to hypoxia was greater in the liver and brain than in the lung and muscle during the final days of egg incubation. The observation suggests that HIF-1α has a role in the adaptation of the embryo to hypoxic condition and thus the hatchability of eggs.

Keywords: hypoxia, incubation, chickens, HIF-1α.

S11-0007 The effects of breeder age on yolk unsaturated fatty acid content, embryo development and chick quality in broilers

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The aim of this study was to investigate the effects of the breeder age on yolk unsaturated fatty acid content, embryo development and chick quality in broilers. In the study, eggs were obtained from 36 and 52 wk old broiler breeders. The unsaturated fatty acid content of the yolk before and during incubation were analyzed. Yolk sac weight, embryo weight and length were measured on day 18 and chick weight and length were measured at hatch. It was found that breeder age significantly affected unsaturated fatty acid content of fresh yolk. In the study, palmitoleic, oleic, linoleic and linolenic acids were higher in 36 wk of age, whereas arachidonic acid was higher in 52 wk of age group. Embryo or chick weight and length, and yolk-free body weight were found to be higher in embryos from 52 wk of age group during incubation and at hatch. Chick weight and length 40.4 g and 19.6 cm, 47.8 g and 21.0 cm in chicks from the 36 and 52 wk of age groups, respectively. At hatch, palmitoleic and oleic acid in the residual yolk sacs of embryos from breeders of 36 wk of age (1.82 and 32.68%, respectively) were higher compared to 52 wk of age group (1.30 and 29.93%, respectively; $P < 0.01$). On the other hand, linoleic and arachidonic acid concentrations were found to be higher in the residual yolk sacs of embryos from those of 52 wk of age (13.90 and 5.74%, respectively) compared to 36 wk of age group (13.55 and 4.57%, respectively; $P < 0.01$). In conclusion, broiler breeder age affected unsaturated fatty acid content and embryo development during incubation and also chick quality at hatch.

Keywords: breeder age, unsaturated fatty acid composition, embryo development, chick quality

S11-0008 Characterization of the genetic mechanisms responsible for adaptation to hypoxic stress during broiler embryogenesis

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Environmental alteration during development of an organism may alter development of some physiological regulatory systems and induce permanent phenotypic changes in the chick embryo. Hypoxia during the incubation of broilers was found to have an effect on the physiology and metabolism of the embryos. The purpose of this study was to investigate the effect of hypoxic stress of 17% oxygen during the plateau stage of oxygen consumption on broilers embryonic development, heart tissue gene expression and understand the genetic mechanism that allows chick embryos to cope with the hypoxic stress. RNA was extracted from hearts of commercial broiler embryos that were subjected to 17% hypoxia on embryonic day 16 (E16). The samples were collected after 2, 8 and 12 hours of hypoxia. The transcriptome of the hearts was formed after RNA-seq and expression of the genes was compared to a control group in order to find pathways that contribute to the embryos' adaptive response to hypoxia. In 530 genes, differential expression patterns were identified between hypoxic and control hearts in all three time points. Those genes were associated with many different pathways including response to stress, regulation of growth, and proteolysis. In two different time points (after 2 and 8 hours of hypoxia), processes occurring in the mitochondria, such as respiratory chain, were found to differ between the hypoxic and control groups. Our findings suggest that the cellular metabolic and respiratory activity in the embryos decrease after 2 hours of hypoxia, and later on (after 8 hours) there was an adaptation, indicated by elevated activity, allowing supply of the energy that was lost due to the first response. Better understanding of the embryos' response to hypoxia, will allow us to determine the exact incubation conditions for optimal programming of the regulatory systems of broiler embryos in order to improve their post hatch performance under sub-optimal conditions.

Keywords: embryogenesis, hypoxia, RNA-seq, incubation, broiler, gene

S11-0009 Effects of incubation conditions on hatchability and hatch parameters in two layer strains

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Suboptimal incubation conditions affect embryo development and hatchability. One experiment was conducted to explore the effects of incubation temperature (INC) profiles on hatchability and organ weights at hatch on two layer strains: Hyline (HL) Brown and Hyline W-36. Two incubation temperature profiles were evaluated the standard incubation profile (S) consisted of maintaining eggshell temperatures at 37.8°C, simulating single-stage incubation. The second profile (LH) had low (36.9°C) eggshell temperature for the first 3 days, and S INC until the last 3 days when eggs were subjected to elevated (38.9°C) eggshell INC simulating multi-stage incubation. Eggshell temperatures were measured 4 times per day with pipe-probes attached to eggs and measured with a thermistor to avoid opening the machine. Hatchability and fertility were calculated at hatch. Additionally 12 pullets per treatment combination were sampled for BW, yolk-free BW, heart, liver, proventriculus, and gizzard weight. Ceca and intestine length were also recorded. Data was analyzed as a completely randomized design with a 2 x 2 factorial arrangement of treatments with layer strains (HL-Brown and W-36) and incubation conditions (Standard and Low-High) as main factors. Hyline W-36 pullets had the higher hatchability % compared to the HL Brown. There was no ($P > 0.05$) difference on fertility due to treatments. The HL-brown pullets had greater ($P < 0.05$) yolk-free BW as well as gizzard and heart weights compared to the W-36, but W-36 pullets had higher liver weights. The only significant ($P < 0.05$) effects of incubation detected on these pullets were observed with the liver and heart weights. Pullets from the standard incubation profile had bigger hearts and livers than pullets from Low-High incubation. It was concluded that genetic lines differed on hatchability and embryo development and incubation temperature profiles had an effect on organ development of pullets.

Keywords: incubation, hatchability, pullets, embryo development

S11-0012 Post hatch development of alkaline phosphatase activity in the broiler small intestine

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Previous studies showed that alkaline phosphatase activity indicated maturity of intestinal cells and had a key role in long chain fatty acids and cholesterol digestion. Therefore, this study was devoted to clarify the changes of alkaline phosphatase activity in the small intestine of broiler chicken throughout the period of breeding. We used a sample of 54 subjects of broilers from hatching period to 56th day of age. The intestinal activity of ALP was spectrophotometrically determined using p- nitrophenylphosphat as a substrate. Experimental data were statistically analyzed while using the t-Student test. Our findings showed a significant increase in the alkaline phosphatase activity at various levels following the different small intestinal segments during the first two weeks of life and then decreased significantly with age ($P < 0.05$). Moreover, alkaline phosphatase activity was higher in the duodenum and jejunum than that seen in the ileum over the period examined. We concluded that alkaline phosphatase as a marker of enterocyte maturation, exhibited high enzymatic activity in the duodenum and jejunum of broilers.

Keywords: alkaline phosphatase, broilers, age, intestine

S11-0013 The effects of pre-heating period on the hatchability and chick development of Ostrich (*Struthio camelus domesticus*) eggs

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This research was carried out with the aim of determining the effects of different pre-heating periods on the hatchability of ostrich eggs and chick development at hatch. A total of 255 hatching ostrich eggs were used in the study. The eggs collected from breeding flock were stored for one week at 16-18°C of storage temperature. The eggs were weighed and then were classified as equal numbers into three experimental groups including three different pre-heating periods as (6 h, 12 h, 24 h-22°C). Hatchability of fertile eggs was found as 64.6, 69.5 and 57.9% for the pre-heating periods of 6, 12 and 24 hours, respectively ($P<0.01$). Similarly, the highest value of hatchability of total eggs was observed for the pre-heating period of 12 h (40.5%, $P<0.01$). In the pre-heating period of 24 h, the highest early and late term embryonic mortalities was found as 15.1% and 22.4%, respectively ($P<0.05$). Chick hatching weight was similar between pre-heating period groups, whereas chick weight/egg weight ratio was observed higher in 6 h and 12 h groups (53.3% vs. 54.9%) than 24 h (45.8%, $P<0.01$). Incubation length was found to be shortest with a value of 1002.8 h in the 24 h of pre-heating group than others (1025.0 and 1034.5 h for groups of 6 and 12 h pre-heating period, respectively, $P<0.01$). In conclusion, extending of pre-heating period caused a decline in hatchability with increasing of early and late term embryonic mortalities and also a decline in chick weight/egg weight ratio.

Keywords: ostrich, pre-heating, breeders, hatchability, embryonic mortalities, incubation length

S11-0014 Relationship between yolk dry matter content and yolk consumption during embryonic development in small and heavy eggs of Peckin ducks

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This study was carried out with the aim of determining the relationship between yolk dry matter content and yolk consumption during embryonic development in small and heavy eggs of Peckin ducks. The eggs were classed into two weight categories as “small” (65-70 g) and “large” (85-90 g). The eggs from each group were sampled to determine fresh yolk weight and dry matter. Yolk sac weight and dry matter were determined on day 14, 21 and transfer day of incubation period to determine the yolk consumption. Also embryo weight and dry matter of embryo and yolk sacs were determined on sampling days (21st day, transfer day, hatching day). Fresh yolk weight increased by a higher ratio in small eggs than large ones ($r=0.970$; $r=0.725$, respectively). On 21st day of incubation, it was found significant correlation between egg weight and yolk absorption in small ($r=0.940$) and large eggs ($r=0.936$), whereas this relationship was only observed in large eggs on transfer day ($r=0.951$). On the other hand, a significant correlation was observed between yolk dry matter and embryo weight for small and large eggs on day 18 of incubation period ($r=0.966$, $r=0.956$). During incubation, significant relationship between yolk dry matter and embryo dry matter was constantly observed for small and large eggs. On hatching day, a significantly negative correlation between egg weight and residual yolk dry matter was observed for small eggs ($r=-0.810$). In large eggs, it was found a negative relationship between chick weight and residual yolk sac weight ($r=-0.896$) and residual yolk sac dry matter ($r=-0.975$). These results show that egg weight in Peckin ducks affects these relationships between yolk dry matter content, yolk consumption and embryo growth during incubation period and hence chick weight at hatch.

Keywords: Peckin duck, egg weight, dry matter, embryo development, yolk consumption

S11-0015 Optimum chick quality is a crucial hinge for optimum performance

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The quality of day old chicks is a corner stone to successful poultry production chain. A study was conducted using 300 locally hatched and foreign hatched broiler day old chicks from day old till 21 days of age post incubation. Analyses were made by euthanizing 25 chicks from each group each week for three weeks and measuring quality characteristics like physical, haematological, immunological, histological and bacteriological parameters. It was observed that on day 1 the live weight of the foreign broiler day old chicks (FBDOC) was greater than the local broiler day old chicks (LBDOC). The chick length and shank length of the FBDOC were also longer than the LBDOC. The navel score of the foreign hatched day old chicks showed higher number of closed navel than the locally hatched day old chicks. In the histological parameters studied, there was little difference between FBDOC and LBDOC in terms of villi length, villi width, villi counts and villi surface area from day old till 21 days. In the haematological/ immunological analysis, red blood cells count of FBDOC were higher than the LBDOC on days 7 and 21. There was also a higher white blood cells count in the FBDOC than the LBDOC. The monocyte percentage was greater in the FBDOC than the LBDOC while the lymphocyte percentage was higher in the LBDOC than the FBDOC indicating varying immunity levels. From day 1 till 21 post hatch *E. coli* was the only isolated bacteria from foreign broiler day old chicks while for the local broiler day old chicks the main bacteria isolate was *E. coli* followed by *Proteus* and *Streptococcus* sp. The LBDOC had a greater wet and dry yolk sac percentage as compared to FBDOC on day one and seven. There was residual yolk sac still present in the foreign and local broiler chicks on days 14 and 21 respectively on dissecting the birds. The results of the study indicate challenging situation in maintaining the quality of locally hatched chicks that needs urgent attention from the local hatcheries.

Keywords: day old chick, foreign chicks, local chicks, residual yolk sac, chick quality

S11-0016 The in ovo application of L-carnitine during embryogenesis in meat-type chicken

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The selection of meat-type chicken for high growth rates led to the impairments in lipid metabolism starting as early as at embryogenesis and leading to increased embryonic mortality during the last days of incubation. L-carnitine is one of the key substances stimulating oxidation of fatty acids to produce energy. The trial was conducted on embryos of meat-type chicken to evaluate the influence of in ovo injection of L-carnitine on the subsequent growth efficiency in broilers. After 18 days of incubation eggs with livable embryos were divided in 3 groups: group 1 (negative control, no injection); group 2 (positive control, single injection of physiological solution, 0.5 ml per egg); group 3 (experimental, single injection with solution of L-carnitine, 2 mg per egg). After the injections all eggs were placed to an incubator for hatch. Hatchability in group 3 was higher by 7.1-7.3% compared to control groups, percentage of undergraded (weak) chicks lower by 3.9%. Live bodyweight in carnitine-injected day-old chicks (38.64 ± 0.23 g) was significantly higher ($P < 0.001$) compared to control group 1 (37.59 ± 0.22 g). The rate of subsequent growth was also higher in carnitine-injected broilers: at day 4 of age live bodyweights in group 3 and 1 were 81.7 ± 1.6 and 76.50 ± 1.7 g, respectively. The differences in biochemical composition at days 1 and 4 of age were found both in body and residual yolk of the chicks. At day 1 concentration of total lipids in body and residual yolk in group 3 was 4.83 and 21.9% respectively while in group 1 5.46 and 22.5%. Concentrations of total protein in serum in group 3 were 26.22 ± 1.35 and $24.24 \pm 2.07\%$ respective to days 1 and 4 of age while in group 1 21.18 ± 1.34 and $18.33 \pm 1.76\%$.

Keywords: broilers, L-carnitine, in ovo injection, live bodyweight, growth rate

S11-0017 Effects of applying a light-dark schedule during incubation on broiler leg bone geometry

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Light-dark rhythms are known to influence embryonic circadian hormone release in chickens. Especially melatonin is darkness-dependent and known to influence bone development. We previously found that a light-dark schedule during incubation stimulated bone growth until slaughter. Aim of the present experiment was to determine how light during incubation influences leg bone geometry in post-hatch broiler chickens. Ross 308 eggs from a 40 week old parent flock were incubated at a constant eggshell temperature of 37.8°C to eliminate any embryo temperature effects. Eggs were kept under continuous light (24L), continuous darkness (24D), or 12 hours of light and 12 hours of darkness (12L:12D) from the start of incubation until hatching. The lights were cool white LEDs providing 500 lux. Post-hatching, chickens were housed under 16L:8D. Between 18.8 and 19.5 days of incubation, blood was collected from 5 embryos per treatment every 6 hours (N = 60) for analysis of plasma melatonin rhythmicity. At hatching (D0), D21, and D35 post-hatch, 54 chickens per treatment (N = 486) were sacrificed. D0 chickens were sampled for GH and IGF-1 analysis. All left and right tibiotarsi and femurs were removed, cleaned, and cut to a 1.8 cm fragment of the exact middle of the shaft using a hacksaw. A hole was drilled into the anterior part of the bone fragment to be able to determine the bone's orientation. Bones were scanned in a Skyscan 1072 micro-CT scanner, images reconstructed in N-Recon, and analyzed using Matlab. Mid-diaphysal cross sections of each bone were used for measurements of outer diameter, cortical area, medullary area, and cortical thickness. Embryonic plasma melatonin levels did not differ between treatments (P = 0.35). Results of GH, IGF-1, and the X-rays will be presented at the conference and will provide insight into influences of light during incubation on post-hatch bone geometry and possibly later life leg health of broilers.

Keywords: incubation, bones, lighting schedules, micro-ct

S11-0018 Effects of genetic line, incubation temperature profiles and gender on the incidence of footpad dermatitis

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Suboptimal incubation affects footpad skin development and can increase the incidence of footpad dermatitis. This experiment was conducted to evaluate the effects of 2 incubation temperature (INC) profiles, genetic lines, and gender on the incidence of footpad dermatitis (FPD). A total of 1,000 eggs from 4 genetic lines: Ross x Ross, Cobb 500 x Cobb MX, Cobb 500 x Hubbard M99, and Athens-Canadian Random Breed Cross, ACRBC (Control) were randomly distributed into four machines with 2 INC profiles. In the first two machines, eggshell temperatures were maintained close to 37.8°C during the whole period to simulate single stage incubation (Standard). In the other two machines, eggshell temperatures were low (36.9°C) for the first 3d and close to 37.8°C until the last 3d when eggs were subjected to elevated (38.9°C) eggshell T, as it is observed in multistage machines (LH). Eggshell temperatures were measured with pipe-probes and thermistors. At hatch, 960 chicks were placed in 80 pens in 5 different rooms with 16 floor pens with used wood shavings for a total of 60 chicks per treatment combination. Chickens were fed starter (0-14d), grower (15-35d) and finisher (36-56d) diets. The FPD scoring was done at 13 and 27 d of age. BW were evaluated at 14 and 28 d of age. Data were analyzed as a randomized complete block design with genetics, incubation and sex as main factors. Both Cobb 500 x Cobb MX and Cobb 500 x Hubbard strains had higher FPD scores (P<0.0001) at 13 and 27 d of age compared to the Ross and the ACRBC strains. The Cobb strains also had higher BW (P<0.0001) at 14 days which indicates that higher BW is associated with incidence of FPD. Chicks coming from the LH incubation always had numerically higher FPD than the standard incubation. It was concluded that these genetic lines differed on live performance and FPD incidence. Suboptimal incubation increased the incidence of footpad dermatitis.

Keywords: incubation, footpad dermatitis, genetic lines

S11-0020 Effects of genetic line, incubation temperature profiles and gender on drip and cook loss on Pectoralis major and minor muscle at 57 d of age

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Meat quality is an important factor in poultry marketing having a direct impact on customer preferences. Drip and cook loss are common meat quality parameters. This experiment was conducted to evaluate the effects of genetic lines, incubation temperature profiles and gender for drip loss and cook loss on Pectoralis major and Pectoralis minor. A total of 1,000 eggs from 4 genetic lines: Cobb 500 x Cobb MX, Cobb x Hubbard M99, Ross 708 x Ross and Athens-Canadian Random Bred Cross were randomly distributed into four machines with two incubation temperature profiles. In two of the machines, eggshell temperatures were maintained close to 37.8°C (100° F) during the whole incubation period to simulate single stage incubation (Standard). In the other two machines, eggshell temperatures were low (36.9°C) for the first 3 days and close to 37.8°C until the last 3 days when eggs were subjected to elevated (38.9°C) eggshell temperatures, as it is observed in multistage setters and hatchers. Eggshell temperatures were measured using pipe-probes and thermistors. At hatch, 960 chicks (12/pen) were randomly distributed into 80 pens with 5 replicate pens/treatment combination. At 57 days, 2 birds per pen were processed and breast drip loss and cook loss on Pectoralis minor and major muscles were calculated. Data were analyzed as randomized complete block design with genetic lines, incubation profiles and sex as main effects. No three-way interaction effects ($P > 0.05$) were detected. No effect ($P > 0.05$) of treatments was observed on drip loss. An interaction effect of genetic line by gender was observed on cook loss for Pectoralis minor ($P < 0.05$) and Pectoralis major ($P < 0.05$) muscles. The greater cook loss on Pectoralis major was detected on males from the Cobb x Cobb strain. The lowest cook loss for Pectoralis minor was observed on the females from the Cobb x Cobb strain. In conclusion, cook loss on Pectoralis major and Pectoralis minor differed among genders and genetic lines.

Keywords: drip loss, cook loss, pectoralis major, pectoralis minor, incubation, genetic line

S11-0021 Goose embryo development from oviposition through 16 hours of incubation

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Goose eggs have a longer incubation period than other poultry species and are subject to a high embryonic mortality rates during incubation. To improve the hatchability of goose eggs, poultry scientists must be able to precisely describe the stage of embryo development under different egg storage and incubation protocols. Therefore, the aim of the study was to examine the stages of early embryonic development of geese at oviposition and through 16 hr of incubation. White Koluda goose eggs were collected twice a day and stored in a cool room (12-15°C) for maximum 3 days. Incubation was performed at 37.4°C and 52% humidity. Blastoderms were isolated from eggs incubated for 4, 8, 12 and 16 hr and staged according to Eyal-Giladi and Kochav (EGK, Roman numerals) and Hamburger and Hamilton (HH, Arabic numerals) procedures. At oviposition, 39% of the blastoderms were at Stage X, and 25% were Stage XI, which represents the initial stage of hypoblast formation. After 4 hr incubation 65% of blastoderms advanced to Stage XIII, which is characterized by complete hypoblast formation. By 8 hr, 46% were in Stage XIII, while 14% had advanced to Stage 4, undergoing gastrulation. With further incubation embryo growth was slightly slower and after 12 and 16 hr of incubation over 70% of embryos were between Stages 2 to 4. The early stages of goose embryo development are morphologically similar to that of chicken embryo, although there are some differences- the Koller's sickle is not visible, goose blastoderms are larger in diameter and develop slightly slower. The use of a staging table for goose embryos provides a standardize method to communicate precise information on the embryo development either during egg storage, trial incubation treatments, approximate time of embryonic death, and to clearly differentiate a fertilized from unfertilized ovum or early dead embryos. These capabilities will be useful to poultry scientists, hatchery personnel, and developmental biologists.

Keywords: goose, embryonic development, blastoderm, embryo staging

S11- 0023 Egg quality, incubation characteristics and subsequent broiler performance: effect of egg weight of Hubbard Classic Broiler breeders

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The objective of the study was to investigate the effects of egg weight on egg quality, incubation characteristics and subsequent broiler performance at 30 week age of the Hubbard broiler breeders. A total of 930 eggs were collected and divided into three egg weight categories small, medium and large having the weight $51.00c \pm 0.36$, $56.07b \pm 0.56$ and $61.06a \pm 0.15$ gram respectively. Ten eggs from each egg weight category was used to determine the egg quality parameters and remaining 300 eggs were used to determine the incubation parameters and post hatch broiler performance. No difference was noted for the shell weight, shell thickness, yolk weight, albumen weight, yolk to albumen ratio, shape index and specific gravity due to different egg weight groups. Highest water loss was observed ($P < 0.05$) for small eggs at 7th, 14th and 18th day of incubation. Fertility and hatchability of large eggs was higher ($P < 0.05$) compared to small eggs. Embryonic mortality of first, second and third week was more ($P < 0.05$) for small eggs than larger ones. Chick length and weight was increased by the increase in egg weight. Body weight of female broiler was significantly higher at 21 day and it remained non-significant for males up to 42 days of their rearing. Feed intake of broilers was not affected by the egg weight categories but feed conversion ratio was higher for female chicks hatched from the small eggs at second and third week. In conclusion egg weight positively affected the chick characteristics (e.g. chick weight, chick length) and did not affect the final live body weight, feed conversion ratio, feed intake and mortality in broilers at early stages of the production of broiler breeders.

Keywords: egg weight, egg quality, chick quality, broiler performance, feed intake

S11-0024 Preliminary study on utilization of screen house and Solar drier as poultry egg incubators

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Traditionally, local hens incubate and hatch their own eggs even others' eggs as foster hens. Unfortunately, most improved poultry birds do not sit to incubate their own eggs hence, the discovery of artificial incubators. With technological advancement, the artificial incubators have become so sophisticated that higher percentage hatchability and chick performance have been reported. However, most prospective farmers especially in the developing countries may not be able to afford this hence, the need to harness the prevailing rise in temperature due to climate change. Thus, this study was aimed at using Screen house and Solar drier as egg incubators. Sixty quail fertile eggs and fifty Shika brown fertile eggs were allotted to three treatments: T1 (Artificial incubator as control), T2 (Screen house) and T3 (Solar drier) with 0, 30 and 30 quail eggs respectively. Whereas 30, 10 and 10 Shika brown eggs were distributed to T1, T2 and T3 accordingly. The eggs in T2 and T3 were kept five each in separate plastic baskets, covered with cotton wool with water container beside them. The incubation temperatures varied between 26°C (night) and 42°C (day) in T2 and 29°C and 42°C (day) in T3 whereas, T1 temperature fluctuated between 36°C and 38°C. It was observed that 11 out of the 30 eggs (representing 36.7%) hatched in T1 and none of the eggs in T2 and T3 hatched. Interestingly, it was discovered that 53% of the eggs in T2 developed up to embryonic stage 9 while 48% of those in T3 developed up to stage 7 according to Hamburger chick embryonic growth and development stages. This observation could be largely due to the irregular turning of the eggs and the decline in temperature at night. Meanwhile, mean incubation temperature at 22°C at certain periods of incubation was reported to hatch eggs even reversed chick sex. Therefore, Screen house and Solar drier if properly prepared, may be utilized as incubators to harness the climate change for biotechnological advantages.

Keywords: climate change, hatchery, poultry species, temperature

S12-0001 Novel Muscovy duck parvovirus outbreak, China

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Beginning in late 2009, ducklings in China began showing abnormal symptoms, such as shortened beaks, growth retardation, and pancreatic and duodenal mucosa bleeding. These symptoms appeared even in ducks immunized with an attenuated live MDPV and GPV vaccine. The mortality rate of 53% caused substantial losses, and even the surviving ducks suffered from shortened beaks and stunted growth. The aim of the presented study was to analyze and characterize this novel MDPV strain, using pathogen detection, virus isolation and identification, sequencing and analysis of the whole genome and animal regression testing of the virus. We found great differences in the genome, host animal range and pathogenesis between this newly identified strain and the classic MDPV strain. Accordingly, we proffer that this strain be classified as a novel MDPV and this study will provide a solid foundation for the future study of genetic variation mechanisms and pathogenesis of waterfowl parvoviruses.

Keywords: novel duck parvovirus, gene recombination, outbreak

S12- 0002 Genomic analysis goose parvovirus isolated from Anser cygnoides

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In this study, we sequenced and analyzed the complete genomes of the goose parvovirus (GPV) isolates (strain FJ01) derived from *Anser cygnoides*. The GPV strain FJ01 genome is 5104 nucleotides (nt) in length and has a basic structure similar to previously reported GPV genomes. The genome is flanked on the 5' and 3' terminal ends by 443 nt inverted terminal repeats (ITRs) regions. The distal 405 nt of each repeat form a U-shaped hairpin structure consisting of a 181 base-pair double-stranded "stem" region and a 43 nt bubble region, which serve as the origin of GPV replication. The sequenced genome has a NS coding region of 1884 nucleotides and a VP1 coding region of 2199 nucleotides. Phylogenetic analysis showed that two types of GPV virulent viruses are circulating in China. The GPV strain FJ01 appears closer to the strain SHFX1201, which was isolated from a swan in Shanghai, China. In summary, this report presents the first evidence that goose parvovirus can infect *Anser cygnoides* directly. Bivalent attenuated vaccine against GPV had been used to prevent GPV infections in geese and duck flocks in China for decades, however whether attenuated vaccine against GPV can be used for *Anser cygnoides* flocks to prevent GPV infection needs further investigation. These findings suggest that the *Anser cygnoides* could serve as a potential host for GPV and enable us to understand the molecular characteristics and evolutionary diversity of GPV.

Keywords: goose parvovirus, *Anser cygnoides*, genome, analysis

S12-0003 Effects of in ovo triiodothyronine administration on duck body growth

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In the experiment, we investigated the changes of development status and related genes expression by injecting exogenous triiodothyronine (T3) into fertilized eggs of slow-growing Jinding ducks. Two hundred duck eggs were divided randomly into control and T3-treated groups. The treated eggs were injected with 250 ng T3 in physiological saline into the albumen before incubation. Control eggs were injected with saline without T3. Serum T3 and tetraiodothyronine (T4) levels were detected by radioimmunoassay, deiodinase I (D1), deiodinase III (D3), insulin-like growth factor 1 receptor (IGF1R) and thyroid hormone receptor α (TR α) mRNA expression levels were evaluated by real-time fluorescent quantitative PCR (RT-qPCR), and D1 and D3 protein levels and enzyme activities in the liver were evaluated by ELISA at embryonic day (eday) 27 and post-hatching day 7. BW and pectoralis major muscle (PEM) weight were both significantly increased in the treated group compared with the control group at eday 27 and post-hatching day 7 ($P = 0.035$, 0.044 for BW, and $P = 0.027$, 0.037 for PEM). T3 or T4 levels in the treated group were both significantly lower than that of control group at day 7 (both $P = 0.040$). D1 and D3 protein contents and activities, and D3 mRNA expression in the liver were all significantly down-regulated in the treated group compared with the control group at day 7 ($P = 0.017$, 0.013 , 0.045 , 0.039 , 0.040 , respectively). At day 7, PEM IGF1R and TR α levels were significantly higher in the treated group ($P = 0.045$ and 0.032), while no difference for gastrocnemius (GAS) TR α between the two groups, and GAS IGF1R level of treated group was significantly lower than that of control group ($P = 0.024$). The results suggest that in ovo T3 administration promotes duck development during the perinatal period. Extrinsic T3 causes declines in circulating thyroid hormone levels and down-regulation of D1 and D3 in the liver after hatching, and promotes IGF1R and TR α gene expression.

Keywords: duck, triiodothyronine, in ovo administration, effect

S12-0004 Effects of dietary methionine levels on choline requirements of starter white Pekin ducks

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A 2×5 factorial experiment, using 2 dietary methionine levels (0.28% and 0.48%) and 5 dietary choline levels (0, 394, 823, 1239, and 1743 mg/kg), was conducted to study the effects of dietary methionine status on choline requirements of starter white Pekin ducks from 7 to 28 days of age. Four hundred eighty 7-d-old male White Pekin ducks were randomly allotted to ten dietary treatments, each containing 6 replicate pens with 8 birds per pen. At 28 d of age, weight gain, feed intake, and feed/gain were measured and the legs of all ducks from each pen were examined for incidence of perosis. Perosis and growth depression were observed in choline-deficient ducks and supplementation of choline reduced perosis and significantly increased weight gain and feed intake regardless of dietary methionine levels ($P < 0.05$). In addition, significant positive effects of dietary methionine supplementation on weight gain, feed intake, and feed/gain were observed at any choline level ($P < 0.05$). Supplementation of 1743 mg/kg choline in diets alleviated the depression of weight gain and feed intake caused by methionine deficiency at 0.28% methionine level. The interaction between choline and methionine influenced weight gain and feed intake of ducks ($P < 0.05$). At 0.28% methionine level, 1743 mg/kg choline group caused 4.92% and 3.23% amount of improvement in weight gain and feed intake compared with 1239 mg/kg choline group, respectively. According to the broken-line regression, the choline requirements of starter Pekin ducks for weight gain and feed intake were 1472 and 1424 mg/kg at 0.28% methionine level and 946 and 907 mg/kg at 0.48% methionine level, respectively. It suggested the choline recommendations of starter Pekin ducks in semi-purified diet were 1448 mg/kg at 0.28% methionine level and 927 mg/kg at 0.48% methionine level, respectively. Compared with the adequate methionine level, methionine deficiency markedly increased the choline requirements of ducks.

Keywords: duck, choline, methionine, growth performance

S12-0005 Effect of feed consumption levels on growth performance and carcass composition during the force-feeding period in foie gras production of male Mule ducks

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In order to avoid excess feed consumption during the force-feeding period in foie gras production, a dose-response experiment with 7 feed consumption levels (450, 540, 630, 720, 810, 900, 990 g/d/bird) was conducted to evaluate the effects of feed consumption levels on growth performance and carcass composition of male Mule ducks from 91 to 102 days of age. One-day-old Mule ducklings (sterile and artificial hybrid of male Albatre Muscovy duck and female Pekin duck) were fed a two-phase commercial diets for ad libitum intake from hatching to 91 days of age, followed by graded feeding levels of a corn diet by force-feeding from 91 to 102 days of age. Fifty-six 91-day-old male Mule ducks with similar body weight were randomly assigned to 7 treatments, with 8 birds per treatment. Birds were housed in individual pens. At 102 days of age, final body weight was measured and body weight gain and feed conversion ratio of ducks from each treatment were calculated from day 91 to 102, and then all ducks were slaughtered to evaluate the yields of skin with subcutaneous fat, abdominal fat, breast meat (including pectoralis major and pectoralis minor), leg meat (including thigh and drum stick), and liver. Significant differences in body weight gain, total liver weight, and liver relative weight were observed among the treatments ($P<0.001$). According to the broken-line regression analysis, the optimal feed consumption levels of male Mule ducks from 91 to 102 days of age for maximum body weight gain, total liver weight, and liver relative weight were 217, 227, and 216 g feed/kg BW $0.75 \cdot d^{-1}$, respectively.

Keywords: ducks, feed consumption level, force-feeding, production performance, foie gras

S12- 0006 Effect of dietary methionine and betaine content on slaughter performance, serum biochemical parameters, and mRNA expression level of BHMT in liver of geese from 21 to 70 days of age

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Methionine (Met) is the first-limiting amino acid in corn-soybean based diet of poultry and plays a crucial role in body protein synthesis in poultry. Betaine (Bet) is a naturally occurring trimethyl derivative of glycine, which donates the methyl group to homocysteine for Met synthesis. Nonetheless, the Met-sparing and fat-distribution effects of Bet are somewhat controversial. Accordingly, this study was conducted to investigate the effects of dietary supplementation of Met and Bet on geese from 21 to 70 days of age. Six Met-deficient diets were prepared according to a 2×3 interaction design that included two levels of Bet (0 and 600 mg/kg) and three levels of Met (0, 600, and 1200 mg/kg). Three hundred 188-d-old healthy male Yangzhou geese with similar body weight were randomly distributed into 6 groups with 5 replicates per treatment and 10 geese per replicate. Slaughter performance were recorded at 70 d of age. Serum biochemical parameters including total protein (TP), albumin (ALB), alanine aminotransferase (ALT), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were measured by using the automatic biochemical analyzer. **RESULTS** Bet supplementation could increase the percentage of eviscerated yield; Increasing supplemental Met led to linear increases in TP, ALB and GLO of geese in serum ($P<0.05$). Bet supplementation significantly increased the concentration of TP, ALB, HDL and LDL in serum ($P<0.05$); Dietary supplementation of Bet increased the mRNA level of the BHMT gene expression of geese ($P<0.05$). Dietary supplementation with Bet would influence carcass and part weights due to its ability to serve as a methyl group donor, which would increase protein synthesis. It is concluded that betaine supplementation (600 mg/kg) had apparent sparing effect on methionine needs for slaughter performance, serum biochemical parameters and BHMT gene expression.

Keywords: betaine, methionine, geese, slaughter performance

S12-0007 Effects of corn starch residue growth on performance, body measurements, slaughter performance and viscera indices of Yangzhou geese at the age of 28 to 70 days

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Corn starch residue is the by-product of corn starch production. It consists with an average nutrients of 17.83 MJ/kg (GE), 25.45 % (CP) and 7.54 % (CF). On one hand, corn starch residue used as diet can conserve feed resources and reduce environment pollution. On the other hand, Corn starch residue is the ideal product to replace parts of the corn and soybean meal. A total of three hundred 28-day-old healthy male Yangzhou geese with similar body weight (895.5 ± 5.5 g) were selected and randomly assigned to five groups including a control and four treatment groups. Each group had six replications (ten geese per replication). The control group was fed with a basal diet and the treatment groups were fed diets supplemented with 10%、20%、30% and 40% corn starch residue (every 10 % corn starch residue replaced 7.28 % corn and 2.72 % soybean meal), respectively, during the trial period(28 to 70 days). Feed intake was measured by pen on a daily basis and body weight was recorded biweekly. RESULTS The ADG and F/G of Yangzhou geese at 28 to 70 d of age was significantly affected by level of corn starch residue in the way of quadratic curves ($P < 0.01$), i.e. ADG was increased firstly and then decreased, whereas F/G was decreased firstly and then increased. Corn starch residue levels did not affect ADFI ($P > 0.05$). Increasing of corn starch residue led to linear decreases in breast length、body length and percentage of abdominal fat of geese ($P < 0.05$). Conversely, increasing of corn starch residue led to linear increases in percentage of leg muscle、intestinal weight index and relative index of geese ($P < 0.05$). Corn starch residue level over 20% decreased the growth and slaughter performance, body size and relative weight of viscera. Considering the profit (income - feed cost), 10% to 20% of corn starch residue was proper to be added to the diet of geese from days 28 to 70.

Keywords: corn starch residue, geese, growth performance, slaughter performance, relative weight of viscera

S12-0008 Effects of dietary fiber on growth performance, slaughter performance, and nutrient utilization in Yangzhou geese

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As herbivorous poultry, geese like to eat grass, green vegetables, and other plants rich in crude fiber. Geese have a very strong ability to digest dietary fiber. In contrast to chickens and ducks, geese have a minimum requirement for dietary crude fiber. We hypothesized that the geese depend on dietary crude fiber and that a lack of dietary crude fiber leads to poor performance. Our results help clarify the importance of dietary fiber for geese and provide a basis for further study of the effects of crude fiber in poultry diets. A total of 468 1 - day - old healthy male Yangzhou goslings with similar body weight were randomly divided into 3 groups with 6 replicates per group and 26 geese per replicate. The geese in groups I and III received diets with 2.5% and 6.1% dietary fiber, respectively, for the entire 70 days. The geese in Group II received a diet with 4.3% dietary fiber for the first 28 days and 6.1% dietary fiber for the next 42 days. The experimental diets were formulated mainly according to the NRC (1994) and prior research results from our laboratory. The geese fed with low-fiber diet had lower body weight, lower average daily gain, higher ratio of feed to gain, lower slaughter yield (semi- eviscerated carcass yield, eviscerated carcass yield, and breast yield), and lower utilization of energy and crude protein compared with other two groups($P < 0.05$ for each comparison). A low-fiber diet had negative effects on growth performance, slaughter performance, serum biochemical parameters, and nutrient utilization in Yangzhou geese from 1 - 70 days of age. The low-fiber diet thus failed to meet the nutritional requirements of the geese. As herbivorous poultry, geese depend on dietary fiber for normal performance. Dietary fiber is thus an essential nutrient for geese.

Keywords: dietary fiber, growth performance, slaughter performance, nutrient utilization, Yangzhou goose

S12-0009 Identification of 2'-5'-oligoadenylate synthetase-like gene in goose: gene structure, expression patterns, and the immune responses against DTMUV

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2'-5'-oligoadenylate synthetase-like (OASL) is a kind of antiviral protein that induced by interferon, which plays an important role in the IFN-mediated antiviral signaling pathway. Here, we cloned and identified 2'-5'-oligoadenylate synthetase-like from the blood of Chinese goose (Sichuan white goose, *Anser cygnoides*) for the first time. The degenerate primers of goose OASL (goOASL) were designed based on the conserved regions of *Gallus gallus* OAS*B and predicted goose OASL, based on the partial sequence obtained, the full-length cDNA sequences of goOASL were gained using 5' and 3' rapid amplification of cDNA ends (RACE) PCR. GoOASL including an ORF of 1527bp, encoding a protein of 508 amino acids with a predicted molecular weight of 58.09 kDa. The tissue distribution profile of goOASL in 2w old gosling and adult goose were identified by Real Time quantitative PCR, which revealed that the highest level of goOASL mRNA transcripts were detected in the blood of adult goose and gosling. All statistical analysis were performed in GraphPad Prism using unpaired two-tailed t-tests. Five three-day-old goslings were each injected with duck tembusu virus (DTMUV), the mRNA transcripts level of goOASL was upregulated in all tested tissues such as cecal tonsil, small intestine and spleen compared with control groups injected with PBS solely. Furthermore, using the stimulus including LPS, R848, Poly (I: C), and ODN2006, as well as the viral pathogens including Goose parvovirus (GPV), H9N2 avian influenza virus, DTMUV to treat goose peripheral blood mononuclear cells for 6h, goOASL mRNA transcripts level was significantly up-regulated in all treated group. Our research data will shed some light on the antiviral immune function of OASL in aquatic birds.

Keywords: goose, OASL, tissue distribution profile, immune responses, duck tembusu virus

S12-0010 Green fodder influence fatty acid composition in Wanxi white goose

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Fatty acid composition (FAC) in goose can be affected by the addition of green fodder (GF), especially polyunsaturated fatty acid (PUFA) content and proportion. In the present study, FAC from abdominal fat of Wanxi White geese was determined in order to study its relationship to GF. A total of 240 geese at 70 d of age with similar body weight were randomly divided into 4 groups, group 1 with basal diet, group 2 to 4 fed with basal diet plus GF containing DM equivalent to 15%, 30%, and 45% concentrate feed. Abdominal fat was collected at 80, 90, and 100 d of age. Geese were weighed and euthanized after 12 h fast. Abdominal fat were harvested and lipid was extracted by Folch's method, esterified with methyl alcohol (93%) containing HCL (3%), then applied to DEGS column and were detected with FID by gas chromatography. Proportions of fatty acids are reported as percentages of total FA by mass. One-way ANOVA was used to test the effects of forage on FAC. Fatty acids detected in abdominal fat mainly composed of palmitic acid (c16), palmitoleic acid (c16:1), stearic acid (c18), oleic acid (c18:1), linoleic acid (c18:2), and α -linolenic acid (c18:3), and the ratio for each one in geese fed with basal diet was 23.11%, 2.40%, 7.39, 48.11%, 18.52% and 0.76%, respectively. There was no significant change of FAC after fed with different content of GF for 10 days. The c16, c18:2, and c18:3 significantly increased in geese fed with 15% to 45% GF for 30 days ($P < 0.05$), and the c18:1 significantly decreased ($P < 0.0001$). While the FAC showed no significant difference among groups fed with certain content of GF ($P > 0.05$). Body weight in geese fed with 30% GF was 3663 g, which was significantly higher than that of geese fed with basal diet or 15% GF ($P < 0.05$). It is clear that GF could increase the content of PUFA in abdominal fat of goose. Considering the body weight and the ratio of linoleic acid and α -linolenic acid, 30% addition of GF could be used for fatten with 30 days in geese.

Keywords: Wanxi white goose, green fodder, fatty acid composition, polyunsaturated fatty acid

S12-0011 Using ultrasonic technology to measure the fatness in Peking ducks and their correlation with carcass traits

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Skin fat characteristics and meat yield are the major objectives for roast-type duck breeding. However, skin and abdominal fat content and meat yield are difficult to be measured using traditional method. The application of vivo real-time ultrasonic (RTU) technology improves measurements of body yield with accuracy and relatively easy in poultry production. Here, 209 6-weeks-old Peking ducks were selected and breast thickness, skin fat thickness were measured by RTU to study the application in duck breeding. The ducks were slaughtered, body weight, body size traits and carcass traits were recorded as the standard methods. We also used RTU to measure the breast muscle thickness in live and measure skin thickness after slaughter. The coefficient of correlation between percentages of body component and breast muscle thickness, skin and fat thickness were calculated. The partial regression analysis were also performed, based on the correlation among body weight, body size traits, thickness traits and carcass traits. The results showed that breast thickness, skin fat thickness of 6-week-old Peking ducks had the significantly positive relation with percentages of corresponding body component ($P<0.01$). Four optimum multiple regression equations were established (F-test, $P<0.01$). The results illustrated the RTU was valuable in the duck breeding and is an effective measurement for breast muscle thickness, skin and fat thickness.

Keywords: Peking ducks, real-time ultrasonic, carcass traits, correlation analysis, regression analysis

S12-0012 Goose parvovirus in Peking ducks, China, 2014-2015

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Since January 2014, a viral infection, characterized by strong growth retardation and smaller beak with protruding tongue, occurred in commercial Cherry Valley Peking ducks, northern China. For the purpose of diagnosis, the body weight and beak size data were measured and analyzed for 10 typical cases and 10 apparently healthy ducks collected from a 44-day-old diseased flock. Subsequently, molecular detection, virus isolation, experimental infections, virion purification and agar gel precipitin (AGP) test were performed. The body weight (1.72 ± 0.29 kg) and mean length of beaks (5.03 ± 0.57 cm) of the typical cases differed significantly from those (2.93 ± 0.26 kg and 7.22 ± 0.43 cm) of the apparently healthy ducks, suggesting that the disease was associated with short beak and dwarfism syndrome (SBDS) of mule duck. Using a pan-waterfowl parvovirus PCR assay, seventy samples, collected from seven provinces in China, were tested positive for goose parvovirus (GPV). Phylogenetic analyses of partial VP1, VP3, full-length NS and VP1 sequences revealed that the GPV strains in this study were grouped to a distinct clade of GPV-related group with previously known GPVs from SBDS of mule duck. Distinctly spherical virus particles without envelopes were observed in the electron microscope, with a diameter of 20-24 nm. A GPV strain (designated JS) was isolated in 10 day-old embryonated goose eggs. The signs typical of SBDS could be reproduced by inoculation of the JS isolates in two-day-old Peking ducklings. The JS isolate exhibited closely antigenic relationship with classical GPV in AGP test. These investigations demonstrated that the etiological agent of SBDS in Peking ducks is a GPV variant belonging to the West-European lineage of goose parvovirus.

Keywords: Cherry Valley Peking duck, Short beak and dwarfism syndrome, Goose parvovirus

S12- 0013 The utilization of lipase supplementation and ultrasonication treatment in feather down washing procedure improvement

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The objectives of this study were to integrate lipase supplementation and ultrasonication treatment into the feather down washing procedure to improve the washing efficiency and reducing the washing wastewater production. In the first experiment, the compatibility of two commercial lipase (Lipolase 100L and Novozyme CALB- L) with different feather down washing detergents were evaluated, the effect of reaction time and lipase supplementation sequence on lipase activity were determined in the same washing condition. Lipolase 100L was applied into the following lipase loading and reaction temperature washing assay with duck's feather down contained high or low fat. The Lipolase 100L showed good compatibility with all kinds of detergents, the higher temperature washing process improved the fat removing efficiency of lipase-detergent mixture. The selected lipase maintained high activity when it supplemented in the soaking process before detergent addition. According to the washing result, it indicated that the optimal washing condition was 40 to 45 degree C with 0.5% Lipolase 100L supplementation, over 65% of the fat on feather down surface was removed under this condition. The second experiment investigated the utility of ultrasonication treatment in the lipase-detergent mixture washing process. After the optimal ultrasonication power was determined, the fat removing ability and product recovery rate under different treatment sequence of detergent, lipase and ultrasonication supplementation were examined. Combining the mild ultrasonication treatment (40 kHz) in the last washing process step resulted in the better fat removing efficiency and improved the filling power of feather down product, even the lipase and detergent under lower supplement level. It suggested that lipase and ultrasonication treatment could be benefit to improve the washing performance of feather down under less detergent and water requirement process.

Keywords: lipase, ultrasonication, feather down, washing procedure

S12- 0014 Effect of pre- incubation on hatchability of Pekin duck eggs different storage time

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Introduction: In breeder farms and hatcheries sometimes duck eggs need to be stored for longer than two weeks. An experiment was carried out to evaluate the effects of every three days pre- incubation 2 hours of storage 1, 2, 3 or 4 weeks Pekin duck eggs on embryo mortality and hatchability. **Materials and Methods:** A total number of 11,320 eggs were collected for four weeks. Weekly collection of eggs to a treatment group and each group set up three repeats. Eggs were stored in a chamber at 12°C and 75% relative air humidity. The every week collect eggs every three days pre- incubation 2 hours at temperature of 37°C and relative air humidity of 60%. After heating, eggs were kept at room temperature for one hour. On the last day of collection, all the eggs were set on their sides in an electric incubator and according to the manufacturer's instructions. **Results:** After pre- incubation process, the egg fertilization rates no significant difference among the four groups. The hatching rate of fertilized egg were 90.09%±0.73 (1th week collection), 94.54%±1.87 (2th week collection), 93.90%±0.72 (3th week collection), 94.70%±0.28 (4th week collection), respectively. The embryonic mortality were 2.60%±0.59 (1th week collection), 1.48%±0.77 (2th week collection), 1.91%±0.21 (3th week collection), 1.66%±0.09 (4th week collection), respectively. After pre- incubation process the 1th week collection eggs hatching rate lower than other three groups ($P < 0.05$) and the embryonic mortality higher than other three groups ($P < 0.05$). **Discussion:** Eggs were heated 2 hours every three days, and were subsequently stored at 12°C, thereby ensuring that the embryo development stage achieved by heating was maintained throughout storage. After pre- incubation process eggs can storage three weeks, and hatching rate can reach 94%, therefore its use may be indicated in commercial operations.

Keywords: Pekin duck, pre-incubation, hatch

S12- 0016 Feeding effects of duckweed (*Lemna minor*) on growth performance and gastrointestinal tract development of Nageswari ducks

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Duckweed; *Lemna minor* (DW) is an important protein, fat and mineral rich feed resource for many fish and birds, especially for ducks. The influence of DW meal in ducks is not clear yet. Duck is a waterfowl and has a different physiology than other poultry. Therefore, a study was carried out to find out inclusion level and effects of DW meal on growth performance and gastrointestinal tract (GIT) development of Nageswari ducks (*Anas platyrhynchos*). Randomly selected 8 wks old female Nageswari ducklings (N=252) were equally divided into four dietary groups (A to D). Four isonitric and isocaloric experimental diets were formulated, where DW meal were used 0, 3, 6 and 9% of the diet A to D, respectively. Each dietary treatment had 3 replication (n=21) and respective feed was offered to the ducklings ad-libitum for the duration of experiment (22 wks). All experimental ducklings were reared in separate compartments, and each compartment consisted of three parts: inside house area, adjacent open area and a connecting water area. Weekly growth rate, FCR, age at first lay and GIT development were studied. The results showed that there were no significantly different ($P>0.05$) in average weekly gain, feed intake and FCR but age at first lay had the significantly different ($P<0.05$) among the dietary treatment. DW meal increased the ratio significantly ($P<0.05$) of crop to live weight, gizzard to live weight, caecum to live weight, the caecum index of ducks at 22 wks age. Villus height in duodenum and jejunum of ducks increased significantly with the increase of DW meal levels ($P<0.05$). Crypt depth in duodenum and jejunum of ducks decreased significantly with the increase of DW meal levels ($P<0.05$). This experiment showed that feeding of DW meal to growing layer ducks could improve GIT growth and small intestinal morphology without effect on performance. This experiment provides evidence that DW meal could have any active elements to enriched GIT of ducks.

Keywords: ducks, duckweed, *Lemna minor*, GIT, performance

S12- 0017 Improvement of indigenous ducks of Bangladesh: performance of first generation comparing with foundation stock

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Selection is one of the vital tools for improving the indigenous duck genetic resources. This research is a part of the long-term selection program being undertaken with the objective to compare the performance of three indigenous duck genotypes under intensive management. A total of 1236-day-old ducklings comprising of 3 types of ducks namely Rupali (R), Nageswari (N) and Non-descript indigenous (NI) were hatched for this study from foundation stock. In foundation stock, selection was practiced at 50 weeks of age, on the basis of an index comprising the parameters of age at sexual maturity (ASM), body weight (BW), egg production (EP) and egg weight (EW). Data were analyzed following general linear model, univariate procedure by SPSS-20 version. Significantly ($P<0.05$) highest fertility (73.66%) and hatchability (71.51%) were found in R genotype. Significantly ($P<0.005$) highest BW of day-old ducklings and adult BW at 50 weeks age were found in NI genotype than others R and N genotypes. There was a non-significant ($P>0.05$) variation in FCR among the genotypes. NI genotype (4.37%) had non-significantly ($P>0.05$) higher chick mortality than R (3.81%) and N (4.19%) at brooding period (0-4 weeks). Significantly ($P<0.05$) higher dressing% was found in R (61.43) genotype than N (60.34) and NI (59.19) genotypes. Significantly ($P<0.01$) higher egg production (25-50 weeks) among the selected group was found in R genotype than N and NI genotypes but ASM was highest in N genotypes. Significantly ($P<0.001$) highest EW, shape index and Haugh unit were also found in R than the N and NI genotypes. As a result of selection, egg production was improved 0.82, 0.72 and 0.91%, respectively for R, N and NI genotypes. In terms of productive and reproductive traits R was superior to the others two genotypes except BW and ASM. These findings of the study also give an impetus for continuing the research for more generations to fulfill the objectives of the long-term program.

Keywords: indigenous ducks, selective breeding, genotype, performance

S12- 0018 Molecular identification and comparative transcriptional analysis of Myxovirus resistance GTPases (Mx) gene in goose (*Anser cygnoide*) after H9N2 AIV infection

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Interferon (IFN)-induced myxovirus resistance GTPases (Mx) belong to the member of dynamin-like GTPases and control a diverse range of viruses. In the study, the obtained goose Mx (goMx) mRNA is 2112bp length and shares partly conserved exons and highly conserved domains with other homologues, characteristic of an GTPase domain (G-Domain), middle domain (MD) and GTPase effector domain (GED). It is suggested that the 629th amino acid (AA629) residue of goMx is serine (Ser) which is relative to the AA631 residue of chicken Mx responsible for lacking antiviral activity. In addition, the goMx AA142 residue in the dynamin family signature distinguishes from other definitely functional Mx. Transcriptional analysis revealed that goMx was mainly expressed in digestive, respiratory and immune systems in a age specific pattern. As revealed that the transcription level of goMx in goose peripheral blood mononuclear cell (PBMC) could be significantly upregulated by various agonists and avian viruses. Furthermore, the time course study of the effects of H9N2 avian influenza virus (AIV) on goMx expression in the infected gosling suggested that H9N2 AIV did change the goMx expression in an irregularity way. In particular, the significant changes of Mx expression were observed in the trachea, lung, small intestine of infected birds. Collectively, goMx protein might maintain its broad antiviral activity by changing a few amino acids at selected sites despite of conserved architectures among Mx proteins from different species.

Keywords: goose; Mx; molecular cloning; tissue distribution profile; H9N2 AIV.

S12- 0019 Analysis of population structure of some allopatry domestic duck groups

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China is a big country with rich duck resources. However, we could not fully utilize local genetic resource due to artificial selection, blindly introduction and other reasons. This study analyzed genetic diversity parameters and group structure data by STR genotyping method in order to reveal the basis of genetic diversity and population structure, to improve conservation methods of allopatry domestic duck groups and provide reference for development and utilization of local duck species resources. In this study, the allele frequency of 12 microsatellite loci in 9 allopatry domestic duck groups were detected by STR genotyping method based on the ABI- 3730XL DNA Sequencing platform. Then the alleles were analyzed by Spss19.0 and Structure2.0 software. The results showed that the polymorphisms of all the 12 loci were abundant and 90 alleles were obtained. The distributions of the alleles were analyzed by principal component analysis (PCA) and 8 principal components were got. The accumulating contribution rate of the first 6 principal components was 88.091%. The genetic differentiation structure of the populations were structured by Structure procedure. The results were consistent with the results of PCA analysis. 12 pairs of selected fluorescence labeling microsatellite primers were used to analyze genetic structure of 9 local duck species. The highly polymorphic primers effectively reflected the genetic diversity information, and accurately and clearly described the genetic relationship between varieties. This study used three-dimensional scatter plots, which is constructed by PCA to quantify spatial distance, explaining the molecular genetic relationship. Duck populations conserved in National waterfowl germplasm resource pool (Taizhou) were analyzed by Structure program, population structure diagram was consistent with the results based on three-dimensional graph of PCA.

Keywords: microsatellite markers, population structure, PCA, structure procedure, domestic duck

S12- 0020 A study on the relationship between eggshell color and eggshell quality in ducks

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The objective of the experiment was conducted to analyze the relationships between eggshell quality and color in ducks by determining its structural and chemical properties. 606 eggs were selected from about 3,289 eggs for measuring eggshell color, strength, thickness and pigment content. The results showed that eggshell thickness and strength of blue eggshell were greater than that of white eggshell in Pekin duck ($P<0.001$). A quadratic tendency in eggshell strength and thickness ($P<0.001$) was observed as the increasing content of biliverdin. However, eggshell thickness and strength of white eggshell in *Anas platyrhynchos* eggs were greater than that of blue eggshell ($P<0.001$). Eggshell strength had a strong correlation with eggshell thickness (Pearson correlation coefficient, $R=0.638$, $P<0.001$), and a low correlation with eggshell color and ($R=0.32$, $P<0.001$). In addition, a low correlation ($R=0.208$, $P<0.001$) between eggshell thickness and color was observed. It was concluded that eggshell quality was related to eggshell thickness, and eggshell color was not a better indicator to predict the eggshell quality.

Keywords: eggshell color; eggshell quality; structural properties; chemical properties

S12- 0021 Identical expression patterns of goose spleens response to GPV and H9N2

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Goose parvovirus (GPV) and avian influenza virus subtype H9N2 is single-stranded DNA (ssDNA) and single-stranded RNA (ssRNA) virus, respectively, both of which can spread in goslings and cause a huge economic loss. To explore the comprehensive transcriptome responses of the goose spleens infected by GPV and H9N2 and to understand the difference between the immune response induced by DNA virus (GPV) and RNA virus (H9N2), high-throughput RNA sequencing (RNA-seq) was performed in the spleens of goslings at 5 day after infected. In the present study, the transcriptome data indicated that the numbers of differentially expressed transcripts in two viruses infected groups were approximately equivalent. Though Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, the up-regulated transcripts in two viruses infected groups mainly involved in immune related pathways, such as complement and coagulation cascades, B or T cell receptor signaling pathway and pattern-recognition receptors signaling pathway. In addition, the fold-changes of innate and adaptive immune genes in two viruses infected groups were similar, which displayed identical expression patterns in innate and adaptive immune pathways, including pattern-recognition receptors signaling pathways, antigen processing and presentation pathway and NF- κ B and JAK-STAT signaling pathways as well as cytokines. Furthermore, most of innate and adaptive immune genes, particularly TLR7, Mx and TRIM25, increased in response to GPV and H9N2 infection, which indicated that the immune response was activated by the two viruses. However, the depression of some genes, such as NF κ B2, IKK α , IKK β , JAK2, MHC II, may be a mechanism by which virus evade the host immune system or a strategy to achieve immune homeostasis.

Keywords: identical expression profile, systemic transcriptome, GPV, H9N2

S12- 0022 Threonine supplementation reduce dietary protein concentration in pekin ducks diet from 1 to 21 days of age

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The present experiment was conducted to evaluate the reduction extent of dietary crude protein with supplementation of threonine (Thr) by investigating the effect of dietary threonine and crude protein levels on growth and carcass performance in Pekin ducks from 1 to 21 days of age. 1200 birds were selected from 1500 birds according to average body weight (BW) and randomly allotted to 1 of 25 dietary treatments with 6 replicate cages of 8 birds per cage for each treatment. Birds were fed with the 5 basal diets with 16, 17.5, 19, 20.5, and 22% crude protein and basal diets supplemented with Thr ranging from 0.00 to 0.28% with 0.06% increment from 1 to 21 days of age, respectively. The 5 basal diets contained 16.13, 17.65, 19.18, 20.81, and 22.29% protein with 0.36, 0.41, 0.46, 0.52, and 0.59% Thr by analysis as dry matter basis. The results showed the BW, average daily feed intake, average daily gain (ADG), and percentage of breast were increased, and the ratio of feed/gain and percentage of abdominal fat were decreased as the increasing dietary protein and Thr. However, the percentage of thigh was not influenced by dietary protein and Thr. The maximum BW at the five protein diet was 1100, 1181, 1223, 1264, and 1239g, respectively. According to the quadratic broken-line model, Thr requirements for maximum ADG and percentage of breast in Pekin ducks from 1 to 21 days of age were 0.66 and 0.67% for 19% protein diet, and 0.69 and 0.73% for 20.5% protein diet, and 0.70 and 0.73% for 22% protein diet. The maximum ADG and percentage of breast were 55.18, 56.90, 55.87 g/day/bird, and 2.79, 2.96, 2.75%, respectively. It was concluded that Thr requirement of Pekin ducks from 1 to 21 days of age was increased as the increasing dietary protein level. The dietary protein levels could be reduced to 19% with the supplementation of 0.21% Thr (total Thr content was 0.67%).

Keywords: threonine, ducks, protein, requirement

S12- 0023 Resequencing ten generations pekin duck Z2 line reveal the genome mechanism of breeding

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Z2 is a female parent of paternal line in Pekin duck multiline cross system bred by our group. Continuous breeding for 10 generations made Z2 line's breast weight increased nearly 3 folds. This is a typical microevolution process accumulated by adaptive genetic variations under artificial selection. But, how many critical genes involved in this microevolution process and how to play their roles? The answers would help us to understand the genomic variation mechanism of breeding. We random selected 30 ducks (15 males and 15 females) per generation of No.1,4, 7 and 10 generation using next generation sequencing (NGS) technology to resequencing and calling the population variation. We identified a map of genome variation encompasses ~10 million SNP and ~2 million indel. Both the principle component analysis and Fst show obvious differentiation between 1 and 10 generation. We found the 5 locus of top 6 regions which most associated with breast weight in genome-wide association study were overlap with top 5% differentiation genome region of Fst analysis. The gene frequency of the 5 locus changed follow an identical trends among the ten generations. Suggested the beneficial allele frequency promoted and accumulated in the population after strong artificial selection, but all of these alleles not fixed in population. That means beneficial allele frequency will return to equilibrium state quickly if the selection stop. Our study elucidated the polygene mechanism of complex trait in the duck breeding process, and help to apply the research achievement to duck molecular design breeding initiatively.

Keywords: pekin duck, breeding, GWAS, Fst

S12-0025 Genome-wide mining and characterization of microsatellite markers in Pekin duck (*Anas platy-rhynchos*)

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Our research was aimed to identify genome-wide microsatellites (Simple Sequence Repeats, SSRs) for assisting genetic study and marker-assisted selection in Pekin duck. Microsatellites were identified by screening the reference genome sequence of Pekin duck using MISA software with default parameters, and a total of 123,164 perfect microsatellites (Di-, tri-, tetra-, penta- and hexa-nucleotide) were detected, with an average density of 11.3 SSRs/Mb. Di-nucleotides were the most abundant category, accounting for 43.6% of the total SSRs, followed by tri-nucleotides (23.8%) and tetra-nucleotides (22.9%). In contrast, penta-nucleotides and hexa-nucleotides were less frequent compared with others. Considering the sequence complementarity, the major motifs were rich in AT/AT, AAT/ATT, AAAC/GTTT, AAAAC/GTTTT, AAAAAG/CTTTTT, and the minor motifs were mostly rich in C/G. The analysis of the genomic distribution of 123,164 microsatellites revealed that more than 80% of SSR sequences were commonly mapped onto intergenic and intronic regions. However, only 507 and 209 sequences were annotated to exonic regions and UTR, suggesting a high selection pressure among these regions. In order to obtain the genome-wide microsatellite markers, a total of 89,565 (72.7%) primer pairs were designed from flanking sequences (200 bp) of identified microsatellites with PRIMER3 software, and through electronic PCR analysis, a set of 87,909 primer pairs succeed in generating specific amplicons were extracted as candidate microsatellite markers for further study. Consequently, these genome-wide microsatellite markers were useful in genotyping applications such as germplasm characterization and high-density microsatellite marker linkage map construction in Pekin duck.

Keywords: genome sequences, microsatellite, duck

S12-0026 Effects of ramie on growth performance, slaughter performance of Yangzhou geese at 6–11 weeks of age

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Fodder ramie (*Boeheria nivea*L.) belongs to urticaceae (ramie) fiber crop. One of the special fiber crops in China. Under the conditions of high temperature and high humidity, ramie can still grow normally, and high biological production can be obtained. The content of crude protein of ramie is high. The ramie has reasonable nutrition structure, contains high content of crude protein and a variety of vitamins and amino acids. On one hand, ramie is a fiber crop. On the other hand, it can serve as the feed of herbivorous animals, and provide nutrition for animal growth and development. A total of 300 35-day-old healthy male Yangzhou geese with similar body weight were randomly divided into 5 groups with 6 replicates per group and 10 geese per replicate. The geese in the control group received the basal diet, the geese in the other groups received a diet with 3%, 6%, 9%, 12% ramie. The whole experiment lasted 42 days. In addition, theramie was taken from the third stage of growth period. After natural drying, pulverizing processed into powder (60~100 mesh). RESULTS There was no significant difference among the groups in body weight of Yangzhou geese at 6~11 weeks of age, but the body weight of the geese fed with 6% ramie was the highest. The geese fed with 9% ramie had higher abdominal fat yield compared with those in other groups ($P < 0.05$). The weights of cecum and gizzard in the group fed with 12% ramie were lower compared with the control group ($P < 0.05$). The geese received 12% ramie had no significant difference in growth performance, whereas depressed the development of internal organs compared with the other groups. Taken together the results suggest that the proper addition amount of ramie the third stage of growth period was less than 9%.

Keywords: ramie, growth performance, slaughter performance, Yangzhou goose

S12-0027 Pathogenicity research of short beak goose parvovirus strain (SB-GPV) in Cherry Valley ducks

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Pathogenicity of a new goose parvovirus strain, Short beak goose parvovirus (SB-GPV), in Cherry Valley ducks was described in this study. [Materials] Cherry Valley ducks (2 days of age), SB-GPV M15 strain, anti-SB-GPV antibody, anti-MPV monoclonal antibody, latex-labeled monoclonal antibody (MPVMab15-L and GPVE16-L) and FITC-goat anti-mouse Ig. 30 Cherry Valley ducks were randomly assigned into 3 groups, with 10 ducks in each group. Oral infection group were given 0.3 mL embryo-adapted SB-GPV M15, while control group (feed alone) and live together group (feed with oral infection group) were given 0.3mL Hanks' solution through oral. Clinical symptoms, morbidity and mortality were record every day. Body weight beak length and width were measured at 14, 21, 35 and 49 days post infection (PI) and the data were analysis of variance with the repeated-measurement test for statistical analysis. Anti-SB-GPV antibody and anti-MPV antibody of 5 randomly chose ducks in each groups were detected by LPAI. Dead and stunted ducks at 49 days PI were autopsied. Liver, spleen, kidney, cecal tonsil as well as duodenum sample were sterilely taken for IFA. SB-GPV can infect 2 days old Cherry Valley ducks through oral. Symptoms of artificial infection were similar to the natural infection, which were weak foot, short beak, long tongue, fracture (wing, leg or rib), and dwarfism. Morbidity and mortality were 70%~100% and 10%~30%. The body weight and beak length of stunted duck were 61.1% ~67.6% and 63.1% ~64.8% to healthy duck. 60%~80% ducks showed anti-GPV antibody positive in 7 days after infected, and 100% in 14 days after infected. Even at 66 days after infected, the anti-GPV antibody still kept at a high level. IFA of dead ducks tissue showed strong positive about liver, spleen and heart by using anti-GPV antibody. All the results imply that SB-GPV M15 strain can infect Cherry Valley ducks through oral with high-pathogenicity, which haven't been reported before.

Keywords: short beak goose parvovirus, Cherry Valley duck, body weight, length of beak, pathogenicity

S12-0028 Effects of lighting intensity on seminal and reproductive performance in breeding geese

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This study was to investigate the effects of two lighting intensity, i.e. 300 lux and 40 lux for laying period on seminal and reproductive performance of White Roman geese kept in an environmentally controlled house by using a completely randomized design. The design was comprised of two lighting intensity randomly assigned into the house with 10 pens so that 46 ganders and 136 geese in total were used. The results showed that the geese under 300 lux in comparison with those under 40 lux had no significant in body weight. The egg number of the geese under 300 lux lighting intensity was higher than that under 40 lux (62.6 vs. 45.3 eggs). The laying rate of the geese under 300 lux lighting intensity was higher than that under 40 lux (23.2% vs. 18.9%). The laying period of the geese under 300 lux lighting intensity was longer than that under 40 lux (272 vs. 254 days). In conclusion, there were no significant effect between 300 lux and 40 lux light intensity on body weight, fertility and hatchability of breeding eggs. The higher lighting intensity might increase the egg number, laying rate and laying period of geese.

Keywords: White Roman goose, lighting intensity, reproductive performance

S12-0029 Productivity of Bali ducks fed ration mixed with biosupplement from wastes of the rumen materials of Bali cows

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Development of Bali ducks kept by people in Bali always integrated with agricultural products and animal wastes. This study aims to study productivity on of Bali ducks fed ration base on biosupplement from wastes of the rumen materials of Bali cows. It has been carried out in Peguyangan village, Denpasar, Bali. The ducks were randomly allotted into receive five dietary treatments and three replicates of five ducks/replicate. The four biosupplement produced from wastes of rumen materials of Bali cows as follows, i.e : (a) BWRM0= biosupplement based on wastes from rumen materials fermented without bacteria isolate; 2) BWRM1 = biosupplement based on wastes from rumen materials fermented superior 1 (BWRM3.5) of cellulolytic bacteria culture; 3) BWRM2 = biosupplement based on wastes from rumen materials fermented superior 2 (BWRM3.3) of cellulolytic bacteria culture; and 4) BWRMmix = biosupplement based on wastes from rumen materials fermented combination from superior 1 (BWRM3.5) and superior 2 (BWRM3.3) of cellulolytic bacteria culture. The five treatments were given as follows: (1) T1 = duck fed ration with supplemented BWRM0; (2) T2 = duck fed ration with supplemented BWRM1; (3) T3 = duck fed ration supplemented BWRM2; (4) T4=duck fed ration with supplemented BWRMmix; and (5) T5 = ducks fed basal ration (without supplemented). The results of the study showed that the duck fed T2 has better performance and significant different ($P<0,05$) as compared to the other treatments, meanwhile for variables of body weight, carcass and non carcass from all treatments has not significant different ($P>0.05$). It was concluded that (1) Bali duck fed ration supplemented BWRM1 (T2) can produce good performance and decrease the fat carcass Bali ducks.

Keywords: biosupplements, rumen materials, Bali cows, bali ducks production

S12- 0030 Interbreeding of Pereyaslav geese with heavier breeds

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The aim of the study was the evaluation of growth, meat yields and quality in interbreed hybrids of Pereyaslav geese. This breed conserving in gene pool of Vladimir Research Institute of Agriculture (VRIA) is characterized by low mortality levels in both adult and young birds and good reproductive efficiency; its low live bodyweight and meat yields, however, requires crossing with heavier breeds to obtain commercially important hybrids. Crossings were performed in VRIA. Live bodyweight in hybrid goslings during the entire periods of raising was higher by 6.7%~12.3% compared to purebred Pereyaslav goslings. This difference was the highest (by 9.6%~12.3%) in hybrids of Pereyaslav × Landes breeds. Slaughter and anatomic analysis of carcasses showed that meat productivity in hybrid goslings was also better compared to purebred Pereyaslav goslings. The yield of edible parts in hybrid goslings was higher by 246.2~353.6 g (or 11.5%~15.8%) in males and 171.0~257.2 g (or 9.4%~13.3%) in females compared to purebred goslings. Pereyaslav breed was suggested as a prospective maternal form for crossings with males of Romny, Large Grey, and Landes breeds.

Keywords: Pereyaslav geese, interbreeding, live bodyweight, meat yields

S12- 0031 Effect of light programs on the spontaneous fattening of goose liver without over-feeding

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We have already obtained spontaneous liver steatosis under experimental conditions in Greylag geese without force feeding. Hyperphagia was induced by combining a feed restriction period before ad libitum corn feeding, with an artificial reduction of day length. In the present work, the effect of the light program on the extent of hyperphagia and liver steatosis was investigated. 400 male geese had free access to a standard growth diet from birth (d0) to the age of 105 days (d105). Food access was then restricted to 1h/d until d132. Corn was provided ad libitum from d133 to d217. The birds had access to an outdoor area and were kept under a natural light cycle during the first 15 weeks. Then, this access was interrupted and an artificial light cycle of 10hLight:14hDark (10hL:14hD) was set up until d119. Three light programs were then tested from d120 to d217: kept as 10hL:14hD (group A); 10hL:14hD until d147 and a light reduction of 30 min every 2 days until 7hL:17hD from d161 to d217 (group B); 10hL:14hD until d119 and a light reduction of 30 min every 2 days until 7hL:17hD from d133 to d217 (group C). The feed intake during the first week of ad libitum corn feeding (d133 to d140) was significantly higher in group C than in the two other groups (436g/d/bird vs 343 and 330 in groups A and B, respectively; $P < 0.05$) but over the whole period (d133-d217), there were no significant differences between treatments for total corn consumption (244g/d/bird on average; NS). At d217, the liver weight, which was closely related to liver lipid content, was significantly ($P < 0.05$) higher in birds from group C (321 g) than in birds from group A (248 g), birds from group B showing intermediate values (284 g). Overall, the variability in liver weight was high ($CV > 60\%$). The present data confirm that hyperphagia and related liver steatosis are enhanced by a reduction of day length. This effect is maximized when the shortest light duration and the corn delivery start at the same time.

Keywords: liver steatosis, spontaneous fattening, geese, hyperphagia, light cycle

S12- 0032 Comparison of the environmental impacts of the goose fatty liver produced using over-feeding or spontaneous fattening

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To follow the UE recommendation, we work to develop an innovative livestock production system to obtain liver steatosis in Greylag geese (*Anser anser*) without overfeeding. However, a new production system must not only be technically feasible and socially acceptable, it must also be environmentally friendly. The aim of this study was to compare the environmental impacts associated with the production of goose liver fattened spontaneously or using overfeeding. We used 280 ganders divided in two groups : control group (C system, $n=100$) in which birds were fed a starter diet (9.3 kg from 1-41 d) and a growing diet (19.0 kg, from 42-97d) before being overfed with a dry ground corn and water mash (14.3 kg of dry corn from 98-114 d) until slaughter (815 g of fatty liver at 114 d); innovative group (I system, $n=180$) in which birds were fed a starter diet (8.9 kg from 1-41 d) and a growing diet (27.2 kg from 42-140 d) before receiving wet corn ad libitum (28.0 kg of dry corn eq. from 141-224d) until slaughter (515 g fatty liver at 224 d). Total mortality was 6 % and 11% in C and I systems. Environmental impacts indicators of 1 kg of fatty liver (functional unit) were estimated using life cycle analysis with economical allocation method (primary data from experiment or survey, secondary data from Ecoinvent base, SimaPro software). The impacts are higher in the I system: global warming potential (140.55 vs 53.02 kg CO₂-eq.), acidification potential (1.74 vs 0.75 kg SO₂-eq.), eutrophication potential (0.84 vs 0.37 kg PO₄-eq.), terrestrial toxicity (0.32 vs 0.15 kg 1,4-DCB-eq.), land competition (142.68 vs 66.74 m² year), energy demand (905.62 vs 406.66 MJ), water use (8.16 vs 3.44 L). This is explained by the lower productivity (kg of feed/kg fatty liver: +138%) and the longer breeding period (+96%) in I system. In conclusion, the new system needs to be improved to increase its technical performance (e.g. liver weight) to conjugate both environmental and social performances.

Keywords: goose, spontaneous fattening, liver steatosis, environmental impacts

S12- 0033 Response to low- protein diets in starter Pekin ducks and subsequent effects during the growing period

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An dose-response experiment with 6 analyzed dietary CP levels (16.86, 17.89, 18.42, 19.20, 19.58, and 20.33%) was conducted to investigate the effects of low- protein diets on growth response of starter Pekin ducks and the subsequent effects on growth performance and carcass traits of ducks during the following growing period. All starter experimental diets were formulated to contain similar dietary energy level and the standardized ileal digestible amino acid profile including Lys, Met, Thr, Trp, Arg, Ile, Val, and Gly. A total of 480 1- d- old male Pekin ducks were divided to 6 experimental treatments with 8 replicate pens of 10 birds. Ducks were fed with starter low- protein diets from hatch to 19 d of age and then with the same standard growing growing diets with 18.40% analyzed CP from 20 to 35 d of age, respectively. At 19 and 35 d of age, the weight gain, feed intake, feed/gain, and the yield of breast meat, leg meat, and abdominal fat of ducks from each pen were measured. During the starter period, as dietary CP decreased, the weight gain and feed intake were not affected ($P>0.05$) but feed/gain increased when dietary CP decreased to 16.86% ($P<0.05$). Moreover, the yield of breast meat and leg meat were not influenced by reducing dietary CP ($P>0.05$) but the abdominal fat increased when dietary CP was below 18.42% ($P<0.05$). On the other hand, when all birds were fed with the same standard growing diets, the starter protein restriction from 20.33 to 16.86% had no effects on growth performance of growing ducks ($P>0.05$) and it is the same for carcass trait ($P>0.05$). In conclusion, based on similar standardized ileal digestible amino acid profile, it was possible to formulate the low- protein diets containing about 18% CP for starter Pekin ducks without adverse effects on their performance and the starter protein restriction had no negative effect on duck performance during the following growing period.

Keywords: ducks, protein, growth performance, carcass yield

S12-0035 Body weight and feed consumption of turmric on male and female Kumbang Janti duck reared intensively

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Kumbang janti ducks as one of germplasm Profini West Sumatra need to be investigated. Information on the physical characteristics Kumbang janti ducks had enough while this study aimed to evaluate the performance of productivity Kumbang janti ducks male and female by reared intensively. The study 1 using day- old duckling (DOD) Kumbang janti 150 males and 150 females were reared in. 2 plots the cage (150 head per cage). The variables measured were weight DOD, weight of age one week and one month old. In the second study, feed consumption, body weight and feed conversion were measured, using duckling unsex 80 head that will be added to the feed additive turmeric. The research result that heavy hatching ducks that DOD, (42.49 ± 4.73) g males and females 43.84 ± 4.57 . At age 1 week, (245.77 ± 38.8) g males and females 220.24 ± 27.93 and the age of one month of weight ducks research into 865.38 ± 35.64 which males and females 846.27 ± 8.34 . Feed consumption Kumbang janti ducks with the addition of turmeric in the diet ranging from 4080,35 to 4253.35 grams / head. While body weight during the study was 1120.65 to 1256.30 grams / head and the kumbang janti ducks feed conversion, gained 3.38 to 3.38

Keywords: body weight, performance, turmeric, intensively, Kumbang Janti duck

S12- 0036 Outbreak of avian tuberculosis in commercial domestic ducks (*Anas platyrhynchos domestica*)

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Avian tuberculosis is a contagious disease affecting various domestic and wild bird species, caused by *Mycobacterium avium*. It is reported extremely rarely in commercial poultry flocks and has not been reported in commercial domestic ducks to date, with domestic ducks reported to be moderately resistant to *M. avium* infection. Here, we report the first outbreak of avian tuberculosis in commercial domestic duck flocks. We made a presumptive diagnosis of avian tuberculosis based on clinical symptoms (chronic emaciation, persistent mortality, and egg-drop) and postmortem findings such as firm nodules in many visceral organs of ducks. It was interesting to note that the gross tuberculous lesions were observed in skeletal muscle and heart, which rarely reported in birds. The histopathological characteristic lesions of granulomas with central caseation necrosis surrounding lymphocytes further supported the diagnosis. Acid-fast bacilli (AFB) were detected in histopathological sections, fecal smear, and pure culture using Ziehl-Neelsen staining. The liver, spleen and kidney of infected ducks were collected and homogenated, treated with 4% NaOH, then centrifugated. The supernatant were cultured on Middlebrook 7H10 Agar plate. All the cultures grew mycobacteria within 5 - 7 days and reach maximal development in 3 - 4 weeks. The *M. avium* pathogen was isolated and further identified by polymerase chain reaction (PCR) based on insert sequence IS901 and 16S rRNA gene. PCR products of IS901 and 16S rDNA nucleotide sequences were analysed. They shared 100% and 99% similarity with *M. avium* isolates deposited in GenBank, respectively (GenBank accession no. KU145127 and KU161128). In conclusion, we report the first natural infection of avian tuberculosis in commercial domesticated duck flocks. We highlight that avian tuberculosis not only has economic significance for the duck industry, but also presents a zoonotic hazard to humans.

Keywords: avian tuberculosis, *Mycobacterium avium*, 16S rRNA, IS901, Ziehl-Neelsen staining

S12- 0037 Comparative genomic analysis of *Riemerella anatipestifer* CRISPR-Cas systems

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Riemerella anatipestifer (RA) is the pathogen of *Riemerella anatipestifer* infection, which is a major disease confronting the duck industry worldwide. CRISPR-Cas system provides adaptive immunity against exogenous nucleic invasion in prokaryotes. And the CRISPR array also exists in genomes of RA. To date, there is no report on comparative analysis of RA CRISPR-Cas systems due to lack of available genomic data. To analyze the structure and components of CRISPR-Cas systems in RA, we performed sequence analysis of CRISPR-Cas systems in 25 RA strains. We isolated the 19 clinical strains from liver or brain of sick ducks from China. The genome sequences of these isolates were acquired by high-throughput sequencing and assembled by MicrobeTrakr plus v. 0.9.1 (incorporates Velvet v. 1.2.09). Other genome sequences of RA were gained from NCBI genome database. CRISPRfinder, CRISPR Target, and other bioinformatical softwares were applied to analyze the characteristics of RA CRISPR-Cas systems. We found that most of RA strains (20/25) have two CRISPR loci (CRISPR1 and CRISPR2), one possesses cas genes, and the other is orphan. The rest of strains only have one locus, CRISPR1 or CRISPR2. The distance between CRISPR1 and CRISPR2 differs from strains. The length and content of consensus direct repeat sequences as well as the length of spacer sequences of the two loci are different. Only three cas genes (cas1, cas2 and cas9) locate in upstream of CRISPR1 which possesses 47-bp-long repeat sequences, and these characteristics are similar to that of type II-C subtype of Bacteroidetes. The cas operon of CRISPR1 is relatively conserved except several strains. Spacer organization of CRISPR1 is more divergent than that of CRISPR2, and the strain only with CRISPR2 has more insertion sequences, which suggest CRISPR2 to be inactivated. Additionally, only 8% of spacers (13/153) match phage or plasmid sequences. In general, our data first reveal the features of RA CRISPR-Cas systems.

Keywords: *Riemerella anatipestifer*, CRISPR-Cas system, cas gene, bioinformatics

S12-0039 Cross-species antiviral activity of goose interferons against DPV are related to its positive self-feedback regulation and subsequent ISGs induction

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Interferons are a group antiviral cytokines playing the first defense line in the antiviral immunity. Here, the antiviral activity of goose IFN α and IFN γ against duck plague virus (DPV) were determined. The recombinant goose IFN α and IFN γ were around 20 kDa and 18 kDa, the viral copies decreased more 100-fold and viral titers dropped around 100-fold after DPV-EGFP infection in DEF with pre-treatment by IFN α and IFN γ . The ratio of DPV-EGFP positive cells were downregulated by goose IFN α (3.89%) and IFN γ (0.79%) at 36 hpi when compared to control, which is also in a dose-dependent manner. With the interferon-stimulated gene was the “workhorse” of IFNs in mind, the expression of duck Mx and OASL have a significant upregulation ($P < 0.001$) by IFN treatment for 24 hours. Interestingly, duck cells and goose cells showed a similar trend of an increase of ISGs expression after goose IFN α and IFN γ pretreatment. Another fascinating observation is that the positive feedback regulation of type I IFN and type II IFN by goose IFN α and IFN γ was confirmed in the waterfowl for the first time. These results suggest that antiviral activity of goose IFN α and IFN γ is likely attributed to the induction potency on the levels of downstream interferon induced genes. It will help understand the functional significance of interferon antiviral system in aquatic birds and develop prophylactic and therapeutic approaches against viral disease by interferon.

Keywords: interferon, feedback regulation, DPV, interferon stimulated gene.

S12-0041 A comparative study of chemical composition and sensory traits of livers obtained through over-feeding or spontaneous fattening

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Greylag geese are used for the production of fatty liver, also known as “foie gras”. The conventional production system based on overfeeding is however questioned today for ethical reasons. In previous studies, we have demonstrated that Greylag geese are able to develop variable levels of spontaneous liver steatosis when submitted to a feed restriction period, associated with a reduction of day light length, followed by 12 weeks of ad libitum corn feeding during winter season. In the present work we compared livers obtained from this production system (‘Spontaneous Fattening’ group, SF) to those obtained from the conventional system based on overfeeding (Overfed group, OF) with regards to weight, color, measured by CIE L*a*b, chemical composition, sensory characteristics and consumer acceptability tests, carried out on pasteurized livers selected on the basis of the production system and weight classes: OF from 800 to 1000 g, SF from 600 to 800 g and SF from 800 to 1000 g. The global body fattening did not differ between the two groups but the SF livers were lighter than the OF ones (445 vs 1102 g, $P < 0.001$). They also differed in color as they were darker ($L = 54.8$ vs 69.2 , $P < 0.001$) and had a higher index of red ($a = 9.5$ vs 8.0 , $P < 0.001$) and yellow ($b = 29.8$ vs 27.6 , $P < 0.01$) in the SF group compared to the OF group. Concerning the chemical composition, SF livers contained less fat (53.2 vs 56.6%, $P < 0.001$) but more glucose (45 vs 35 $\mu\text{mol/g}$, $P < 0.001$) than OF livers, whereas neutral liver lipid profiles were similar between geese of the two groups. In the SF group, livers presented lower scores for texture, showed higher color and smell intensities (respectively $P < 0.001$ and $P < 0.05$) and had an increased bitter taste ($P < 0.05$) compared to OF livers. These differences were consistent with the results of the consumer acceptability test showing that SF livers were significantly less appreciated than OF livers on all the measured criteria, regardless of the weight class.

Keywords: Liver steatosis, geese, chemical composition, sensory analysis

S12-0042 Effect of different feeding strategies on the induction of a durable hyperphagia and spontaneous liver steatosis in mule ducks

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Our previous studies showed that Greylag geese can develop a prolonged corn hyperphagia during winter season associated with spontaneous liver steatosis. This study aimed to stimulate a similar behavior in mule ducks. 480 male mule ducks, housed in closed pens of 60 animals each, fed a pelleted diet, were submitted to a gradual time restriction to feed access from 49 to 86 days (d) of age (24h to 15 min of feed access/d) combined with a progressive reduction in day length from 75 to 86 d of age (10 to 7h/d). From 87 d of age animals had access to a 100% corn diet and a 2x2 factorial design was applied with two corn feeding modes (ad libitum “AL” vs progressive “P”) and two corn presentations (whole vs grinded corn). In P groups corn availability was increased by 30 g/d starting at 170 g/d. In AL groups time access to feed was ad libitum then changed at 90 d (2h availability AM) and 97 d of age (1h AM + 1h PM) to stimulate feed intake. 4 treatments of 2 pens each (n=120) were formed. 40 animals were slaughtered before corn feeding, at 87 d of age, and 25 animals per treatment were slaughtered at 94, 101 and 115 d of age to evaluate body and liver fattening. Feed intake and performances at slaughter were similar for both corn presentation types. AL ducks expressed a brief hyperphagia (570±50 g/d) on d 87, followed by a decrease under 300 g/d. The changes in time access to feed induced brief increases in feed intake on d 93 and 97 (369±13 g and 406±35 g respectively). Feed intake of P ducks increased until 97 d of age (406±7 g) then decreased similarly to AL birds. After 101 d of age feed intake reached a basal level (148±38 g/d) regardless of the feeding mode. Regardless of the treatments, liver weight only increased slightly at 94 and 101 d of age compared to 87 d of age (84, 83 and 58 g respectively, P<0.05). This study highlights the rapid adaptation of the mule duck to new feeding strategies and the difficulties to induce a spontaneous liver steatosis.

Keywords: liver steatosis, mule duck, feeding strategies, corn

S12-0043 Identification of goose-origin parvovirus as a cause of newly emerging tongue protrusion syndrome in ducklings

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A recent epizootic outbreak of duck tongue protrusion syndrome (DTPS) in China was investigated. Electron microscopic, genetic, and virological studies identified a parvovirus with a greater similarity to goose parvovirus (GPV) (98% protein homology) than to Muscovy duck parvovirus (MDPV) (93% protein homology). The new virus; provisionally designated GPV-QH15, was found antigenically more closely related to GPV when compared to MDPV in virus neutralization assay. These findings were further supported by phylogenetic analysis showing that GPV-QH15 evolved from goose lineage parvoviruses, rather than Muscovy duck or other duck species-related parvoviruses. In all, two genetic lineages (GPV I and II) were identified from analyzed GPVs, showing GPV-QH15 clustered with two goose-origin parvoviruses (GPVa2006 and GPV1995) and forming a distinctive GPV Ila sub-lineage. Prior investigations have found that two previous viruses, GPVa2006 and GPV1995, infected and caused disease in ducklings. Finally, the structural modeling revealed that GPV-QH15 and its closely related GPVa2006/GPV1995 possessed an identical cluster of receptor-interacting amino acid residues in the VP2 protein, a major determinant of viral receptor binding and host specificity. Significantly, three viruses differed from MDPVs and other GPVs in these positions. Taken together, these results suggest that GPV-QH15 represents a new variant of goose-origin parvovirus that currently circulates in ducklings and causes tongue protrusion syndrome, which has not been recognized previously. This new finding highlights a need for future surveillance of GPV-QH15 in poultry toward better understanding both evolution and biology of this new parvovirus.

Keywords: tongue protrusion syndrome, goose-origin parvovirus, variation

S12-0044 Effect of restricted feeding period and refeeding on local duck's performance

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Local ducks in West Sumatra has the potential of producing eggs and meat. Most farmers keeping ducks extensively by releasing in the area of rice fields. Female ducks were reared as a layer while male duck reared as a meat duck. The increasing number of requests meat ducks causing farmers began to raise ducks as a meat ducks, however the growth rate of local duck too slowly. The objectives of this study was to evaluate the effect of restricted feeding period and refeeding on local duck's performance, feed consumption, body weight gain, feed conversion, carcass and carcass percentage, and blood constituent (erythrocytes, haemoglobin and hematocrit). A total number of one hundred of local ducks two weeks old was used and divided into four treatments and five blocks as replication. The treatments applied were A (ad libitum); B (restricted feeding 45% for 1 week); C (restricted feeding 45% for 2 weeks) and D (restricted feeding 45% for 3 weeks). Ducks were fed ad libitum (refeeding) until 8 weeks old after restricted feeding. The results showed that restricted feeding 45% for 3 weeks were highly significant difference ($P < 0.01$) decrease in feed consumption, body weight gain, feed conversion, carcass and carcass percentage, erythrocytes, leucocytes and hematocrit. However, there were no significant difference ($P > 0.05$) after refeeding on feed consumption, body weight gain, feed conversion, carcass and carcass percentage and hematocrit. Refeeding were highly significant ($P < 0.01$) increase erythrocytes and haemoglobin. The conclusion of the research is the ducks fed ad libitum (refeeding) after restricted feeding 45% for 3 weeks causing improve feed efficiency

Keywords: local duck, restricted feeding , refeeding, performance

S12- 0045 Differential regulation of the innate antiviral immune responses by goose type I , II , III interferons

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Interferons (IFNs) are cytokines with important antiviral activities, which involved in the innate and adaptive immune defense against viral infection. The presence of IFNs form the first line in response to against invasive virus, and are normally considered as a selectable treatment for viral and autoimmune diseases. To further investigate the differences in regulatory mechanism of interferon stimulated genes (ISGs) induced by three types of goose IFNs (IFN α , γ and λ) in goose peripheral blood mononuclear cell (PBMCs), we transfected BHK- 21 cells with pcDNA3.1(+)- IFN α , pcDNA3.1(+)- IFN γ , and pcDNA3.1(+)-IFN λ for 24h, which were further used in treating goose PBMCs for 3h, 6h, 12h and 24h at different dose, respectively. Meanwhile, PBMCs were treated with pcDNA3.1(+) and Poly(I:C) (30ng/mL) for 3h, 6h, 12h and 24h as negative and positive control group. We detected the mRNA expression level of goose ISGs genes (relative to goGAPDH) by real-time quantitative PCR (RT-qPCR), including the positive regulators (goMx, goOASL), the negative regulators (goSOCS1, goUSP18), and three types of goose IFNs (goIFN α , γ and λ). In summary, these data have prompted the suggestion that goIFNs through their cognate receptors induce the down-stream ISG which further contribute to host antivirus effect. It is apparent that goIFNs have a stronger effect on PBMCs at 3h and all goIFNs show a self- positive feedback regulation loop. In addition, we found IFN γ play a critical role as an immune regulator in vitro, and there have no direct regulation between goIFN γ and goIFN λ . However, further investigations are needed, which are necessary to clarify the molecular regulatory mechanism of goose IFN type I, II, III in PBMCs.

Keywords: goose, interferons, ISGs, PBMCs, immune regulation

S12-0046 Gradual reduction of polyunsaturated fatty acids in the development of goose fatty liver

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Non-alcoholic fatty liver disease (NAFLD) occurs in both avian and mammalian species. In general, saturated fatty acids (SFAs) are lipotoxic, while polyunsaturated fatty acids (PUFAs) are beneficial to obesity-associated diseases including NAFLD. Previous studies show that hepatic fatty acid composition is associated with human NAFLD. However, it is unclear that this association also occurs to goose fatty liver. To address this, Landes geese were normally-fed or overfed for different time. Liver weight and its ratio to body weight were both dramatically increased in the overfed geese compared to the normally-fed geese at the end of overfeeding experiment, indicating goose fatty liver was successfully induced by overfeeding. Gas chromatography analysis showed that hepatic PUFAs were gradually decreased in the process of overfeeding, especially linoleic acid and arachidonic acid; in contrast, hepatic SFAs plus monounsaturated fatty acids (MUFAs) were gradually increased, primarily palmitic acid plus oleic acid. In conclusion, the reduction of PUFAs is required for the development of goose fatty liver but not for the protective mechanism against steatosis-related harmful effect. This study thus provides an insight into the mechanism underlying the development of goose fatty liver.

Keywords: fatty liver; fatty acid composition; goose; polyunsaturated fatty acid; saturated fatty acid

S12-0047 Involvement of hexokinase 1 and 2 in goose fatty liver

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The mitochondrial outer membrane protein hexokinase 1 and 2 (HK1/2), the first key enzymes to control glucose metabolic rate, catalyze the phosphorylation of glucose to generate glucose-6-phosphate. Whether the genes are involved in goose fatty liver remains unclear. To address this, we first treated goose primary hepatocytes with high level of glucose or insulin as hyperinsulinemia and hyperglycemia are often associated with non-alcoholic fatty liver disease (NAFLD). Quantitative PCR analysis indicated that HK1/2 were upregulated by glucose/insulin. To determine whether HK1/2 were upregulated in goose fatty liver, 6 Landes geese were overfed for 19 days, while the other 6 geese were normally fed. As expected, fatty livers in the overfed geese were much heavier than normal livers in the normally-fed geese. Moreover, the expression of HK1/2 was significantly induced in goose fatty liver by overfeeding compared to normal liver, which suggested the involvement of HK1/2 in goose fatty liver. We subsequently tested if the upregulation of HK1/2 in vitro could be recapitulated in vivo. For this purpose, 24 Landes geese were overfed with normal diet (n=8) or sugar-supplemented diet (the normal diet supplemented with 20% sugar in weight, n=8) for 19 days. Data indicated, compared to the geese overfed with normal diet, the expression of HK1/HK2 were upregulated in the geese overfed with sugar-supplemented diet. This upregulation was accompanied with the increase of liver weight in the geese overfed with sugar-supplemented diet. In conclusion, HK1/2 are involved in goose fatty liver, and supplementing sugar may promote goose fatty liver. Future work should be focused on the identification of transcription factor(s) that mediates the induction of HK1/2 by glucose and insulin.

Keywords: hexokinase, non-alcoholic fatty liver disease, geese, hyperglycemia

S12- 0048 Effect of feeding systems based on whole corn during the rearing period on performances of over-fed male mule ducks

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To reduce feed environmental and economic costs caused by transport, handling, and processing, the use of whole grains has increased recently in poultry production. The aim of this trial was to compare the interest of 2 feeding systems based on whole corn (loose-mix vs. free-choice feeding system) during the finishing period on performances of ducks. 516 one-day-old male mule ducks were divided into 3 groups (4 pens per group) differing in the presentation of the diet they received between 58 and 88 d of age : group COM received a complete pelleted diet (AMEn 12.1 MJ/kg, CP 15.0%) containing 500 g of corn per kg, whereas the two other groups received a 50/50 mix of whole corn seeds (AMEn 13.9 MJ/kg, CP 7.3%) and protein-rich pellets (AMEn 10.3 MJ/kg, CP 22.7%) mixed in the same feeder (LMF group) or in 2 separated feeders (FCF group). From 89 to 99 d, 72 birds/group were overfed with corn and slaughtered at 99 d to determine weight and commercial value of the fatty livers. Feed intake was measured daily. BW was measured at 58, 88 and 99 d of age. From 58 to 88 d, total feed intake of the FCF group was 8% higher than in the 2 other groups ($P = 0.005$). At 88 d, the BW was higher in the COM group than in the FCF (4,959 vs. 4,778g, $P < 0.001$), the LMF group being intermediate (4874g). During the experimental period, FCR and protein intake were higher in the FCF group than in the 2 others (respectively +32%, $P = 0.02$ and +45%, $P < 0.001$) but the energy intake was similar for the 3 groups (92.6 MJ/duck). After overfeeding (99 d), the BW (6,593g), weight (642g) and commercial grading (80% in the best commercial class) of fatty liver were similar in the 3 groups. The present results suggest that loose-mix feeding during the finishing period using whole corn is a solution to reduce the feed costs. Additionally, the use of locally grown grains could reduce the economic and environmental impacts of duck feeding, reducing the transportation and crushing processes.

Keywords: mule ducks, free-choice feeding, loose-mix feeding, corn, overfeeding

S12-0049 The role of miR-29c in the development of goose fatty liver is potentially mediated by its target genes including COL3A1, INSIG1 and SGK1

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MicroRNAs (miRNAs) can degrade its target mRNA or suppress translation by binding to the 3' untranslated region (UTR) of target mRNA. Using RNA-seq technology, we found that the expression of miR-29c was significantly reduced in the livers of the overfed vs. normally-fed Landes male geese ($n=6$ for each group), suggesting that miR-29c may play an important role in goose fatty liver. To uncover how miR-29c functions in goose fatty liver, we first validated the differential expression of miR-29c in goose fatty liver vs. normal liver by quantitative PCR, followed by prediction of its target mRNAs using online bioinformatics tools. The target genes including COL3A1, INSIG1 and SGK1 were verified by dual luciferase reporter system. qRT-PCR analysis indicated that the expression of these target genes were significantly increased ($P < 0.05$) in goose fatty liver compared to normal liver. Moreover, we treated goose primary hepatocytes with glucose, palmitate and insulin at high dosages as hyperglycemia, hyperlipidemia and hyperinsulinemia are clinical symptoms often seen in patients or animals with nonalcoholic fatty liver disease (NAFLD). Data showed that, compared to the control, the expression of miR-29c was significantly suppressed in cultured cells by both 0.5mM palmitate and 100mM glucose, but the expression of miR-29c was not affected by high dosage of insulin. In conclusion, the role of miR-29c in the development of goose fatty liver was potentially mediated by its target genes including COL3A1, INSIG1 and SGK1, and its expression was regulated by hyperglycemia and hyperlipidemia in the context of fatty liver. Future work should address how miR-29c is regulated by NAFLD-related factors and how its target genes function in the development of goose fatty liver.

Keywords: miR-29c, goose, fatty liver, target genes, mediate

S12- 0050 Effects of corn DDGS on performance, egg quality, oxidative status and yolk fatty acid composition in laying ducks

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The study was designed to evaluate the effects of different dietary levels of corn DDGS in laying duck diets on performance, egg quality, oxidative status and egg yolk fatty acid composition. Longyan laying ducks (1080) with similar BW at 19 wk of age were randomly assigned to 6 dietary treatments, each consisting of 6 replicates of 30 birds. The basal diet (I) was a typical corn-soybean ration while the experimental diets (II to VI) substituted corn DDGS for soybean meal and wheat bran and a small reduction of corn. The level of substitution in diets (II to VI) was 6%, 12%, 18%, 24% and 30%. The experiment lasted for 16 wks. Average egg weight decreased linearly as the level of corn DDGS inclusion increased ($P < 0.001$); Haugh unit and albumin weight decreased as linear response to corn DDGS substitution ($P < 0.05$) and yolk colour, plasma triglycerides and liver malondialdehyde content linearly increased ($P < 0.001$); the proportions of C18:1 and total monounsaturated fatty acids (MUFA) in egg yolk linearly decreased with increasing corn DDGS and many of the key polyunsaturated fatty acids (PUFA) like C18:2 n-6, C20:4 n-6 and C18:3 n-3 linearly increased ($P < 0.001$), but not those of C20:5 n-3 and C22:6 n-3; there were no treatment effects on productive performance, eggshell thickness, strength, activities of antioxidant enzymes and egg yolk cholesterol content ($P > 0.05$). In conclusion, the current study suggests that ducks from 19 to 35 wk, could be fed diets with up to about 18% corn DDGS without effect on the number of eggs produced, egg quality and oxidative status. Increasing amounts of corn DDGS linearly increased egg yolk concentrations of key fatty acids like C18:2 n-6, C20:4 n-6 and C18:3 n-3 and decreased C18:1.

Keywords: corn DDGS, performance, egg quality, oxidative status, fatty acids

S12- 0051 Body weight pattern in Kuttanad ducks (*Anas platyrhynchos domesticus*) over two generations of selection

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An experiment was conducted to develop a meat line of Kuttanad ducks through individual selection for body weight at eight weeks of age. One thousand day-old Kuttanad ducklings (S0 generation) were procured from progressive farmers of Kerala. Top ranking 150 females and 25 males were selected and 1000 ducklings were produced through a pedigreed hatch (S1 generation). Similarly, S2 generation was also taken from S1 through a pedigreed hatch avoiding inbreeding. The mean day-old body weight in Kuttanad ducks in the S0 generation was (40.11 ± 0.11) g, whereas in the S1 generation it was (42.11 ± 0.12) g. The day-old body weight decreased to a mean value of (36.10 ± 0.09) g in the S2 generation. At 4 weeks of age, the mean body weight was 405.79 ± 2.92 , 475.05 ± 4.43 , (439.34 ± 5.71) g, respectively, in the S0, S1 and S2 generation. The mean body weight at 8 weeks of age was (1103.38 ± 4.60) g in S0 generation and the body weight was (1078.95 ± 8.69) g in S1 generation. But the mean body weight increased to (1161.23 ± 9.18) g in the S2 generation. At 10 weeks of age, the mean body weight increased to (1334.07 ± 4.60) g in S0 generation, (1282.12 ± 8.52) g in S1 generation and (1364.61 ± 7.42) g in S2 generation. The mean body weight in the S0 generation at 12 weeks of age was (1387.59 ± 4.43) g, whereas in the S1 and S2 generation, the value increased to (1490.43 ± 6.28) g and (1510.66 ± 7.01) g, respectively. In S0 and S1 generation, the mean body weight was almost similar from day old to 4 weeks of age. But, in S1 generation the body weight decreased at 8 weeks of age but this was compensated in the subsequent weeks as evident from the increased body weight at 12 weeks of age. The same trend was noticed in S2 generation also. The selection for body weight was effective in improving the body weight of Kuttanad ducks.

Keywords: Kuttanad ducks, body weight, selection

S12- 0052 Processing yield in Kuttanad ducks (*Anas platyrhynchos*) at 12 weeks of age in three generations

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An experiment was conducted with the objective of evaluating processing yield of Kuttanad ducks in three generations, S0, S1 and S2. Carcass characteristics of 10 ducks each of either sex were evaluated. In males, the mean slaughter weight was 1348.90 ± 44.21 , 1476.10 ± 35.94 and (1502.50 ± 44.05) g in S0, S1 and S2, respectively. In females the mean slaughter weight was 1186.70 ± 23.14 , 1327.20 ± 28.33 and (1348.10 ± 35.75) g in S0, S1 and S2, respectively. The mean eviscerated weight in males and females were (831.64 ± 27.67) g and (772.54 ± 24.48) g in S0 generation, (949.98 ± 25.53) g and (854.03 ± 24.55) g in S1 generation and (995.87 ± 29.56) g and 906.44 ± 27.40 g in S2 generation, respectively. In S0 generation, the mean eviscerated yield without giblets was 61.97 ± 2.11 per cent in males and 64.98 ± 0.94 per cent in females. The mean eviscerated yield including giblets was 68.57 ± 2.29 per cent in males and 71.90 ± 0.90 per cent in females. The mean eviscerated yield excluding giblets in males of S1 generation 64.35 ± 0.68 per cent while the mean eviscerated yield including giblets was 70.94 ± 0.59 percent. In females the mean eviscerated yield without giblets was 64.29 ± 0.86 per cent whereas the mean eviscerated yield along with giblets was 70.80 ± 0.89 per cent. In males of S2 generation, the mean eviscerated yield without giblets was 66.30 ± 0.70 per cent whereas the mean eviscerated yield including giblets was 73.43 ± 0.62 per cent. In females of S2 generation, the mean eviscerated yield without giblets was 67.25 ± 1.02 and the mean eviscerated yield with giblets was 74.30 ± 0.83 per cent. The eviscerated yield increased from S0 to S2 generation with increase in body weight.

Keywords: Kuttanad ducks, eviscerated yield, giblet

S12-0053 Evidence of genetic-background impacting duck's susceptibility to aflatoxicosis more than climate in terms of mortality and productivity-drops in tropical Indian conditions

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Dietary aflatoxin is a threat to duck industry worldwide, especially in tropics. The study covered 3 distinct periods (6 months) of 2015, i.e. late-Rains (Jul-Aug), autumn (Sep-Oct) and winter (Nov-Dec) to assess criticality of roles of genetics vs climate, on productivity-drops and mortality in naturally-arisen Aflatoxicosis, in an organized Duck farm, in eastern-coastal India: Bhubaneswar (yearly-R.H: 52 to 99%). Weekly data from 1959 adult-layers (40-50 wks age) from 3 breeds: Khaki Campbells, Indigenous (Odisha's native) and White Pekins, mainly for 5 traits, (in percentage): duck-day egg production (DDEP); fertility (FRT); hatchability on total-set (HTES) and fertile-eggs basis (HFES) and mortalities were analysed by SAS (9.3) for deriving significance of these factors. Feeding conditions (18% C. P; 2600K.Cal M.E/Kg) were uniform throughout the study. Aflatoxins @ 10.0ppb in feed were recorded in July (28- 37°C; 72- 99% RH), which peaked at 17.5ppb in early-winter and was lowest (3.3ppb) in late winter. Coinciding this toxin trend; confirmation of aflatoxicosis through P.M lesions, histopathology and elevated liver-enzymes, slump was recorded for all the above traits, with highest weekly mortalities ranging from 1.2 to 6.2% across breeds. Breed differences were significant ($P < 0.05$) for FRT and HFES throughout study period. Variation from seasons significantly influenced drop in HDEP to 16 to 32% and in FRT to 10.5 to 47.0% across breeds, with maximum decline visible in Pekins during autumn. The susceptibility to aflatoxins was in the order:-Pekin> native> Khaki Campbell. Seasonal variation had significant impact on all the traits, except hatchability. It was concluded that: for salvaging acceptable productivity from ducks under low to moderate aflatoxicosis, an anti-toxin strategy should be based on genetics and climatic factors, including a vigilant feeding and healthcare regime fixing threshold of 3.0ppb aflatoxins that tended to go disastrous @ >10.0 ppb level.

Keywords: aflatoxin, duck-breeds, egg-production, genetics, mortality

S12-0054 Rice based DDGS in duck egg production and its quality

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Rice based Distillers Dried grain (DDGS) contains crude protein 64.41% and total energy 4739.73 cal/g of dry sample. The amino acids like lysine and methionine + cystine content were 2.172% and 1.663% respectively. An experiment was carried out with Rice based DDGS in the diet of laying Khaki Campbell ducks from 23 weeks to 44 weeks to study the laying performance and quality of eggs. DDGS in the ration replaced soybean cake at the rate of 0, 10, 25, 50 and 75% on iso-nitrogenous basis. Laying Khaki Campbell ducks of 200 nos. were distributed in five experimental groups with 20 ducks in each replicate. It was observed that total number of egg production was significantly increased ($P < 0.05$) with increased level of DDGS in the ration. The quality of egg in different experimental group indicated that egg length (mm), egg width (mm) shape index (%) and egg weight (g) did not influence with the increasing level of DDGS in the ration. The egg shell weight (g), yolk weight (g) followed the same trend. Although shell thickness indicated higher value with increasing level of DDGS in the ration but it was not statistically significant ($P < 0.01$). Higher values of Hough unit, Yolk index and Albumin index were also observed with increasing levels of DDGS in the diet. It was evident from the experiment that higher levels of Rice-DDGS have better performance on egg production and superior egg quality in ducks.

Keywords: rice, DDGS, laying ducks, egg quality

S12-0055 Rice based distillers dried grain on the performance of growing ducks

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Rice based Distillers Dried grain (DDGS) is rich in both protein and energy. The crude protein content is 61.41% and total energy is 4739.73 cal/g of dry DDGS. The amino acids like lysine and methionine + cystine content were 2.172% and 1.663% respectively. DDGS is the ration was replaced soybean cake at the levels of 0, 10, 25 and 75% on iso-nitrogenous basis. In the starter and grower diet of the Khaki Campbell ducks (0~8 weeks & 9~22 weeks). There were five groups with 20 nos. of day old ducklings in each replicate. Growth studies with starter ducklings (0~8 weeks) indicated that superior body weight gain and feed consumption was observed in 25% replacement group. However, significantly lower body weight gain and feed consumption was observed with higher levels of DDGS in the ration. In the growing ducks (9~22 weeks) significantly higher body weight gain was observed with 10% replacement group of DDGS than control. But upto 50% replacement there were any marked differences of performance of growing ducklings in compare to control. From the results it was evident that in the starter ducklings 5.3% and in the growing ducks (9~22 weeks) 10.6% of Rice-DDGS can be incorporated in the diet with definite advantages.

Keywords: rice, DDGS, growing ducks

S12- 0056 Riboflavin deficiency in maternal diet impairs embryonic development of Pekin ducks

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The objective of this experiment was to investigate the effects of riboflavin deficiency in maternal diet on embryonic development of Pekin ducks. A total of 80 45-week-old breeding female ducks were divided into two groups randomly with 40 ducks per group. The ducks were fed a riboflavin-deficient diet (RD group) and a control diet (C group) respectively, which supplemented with 0 and 10 mg riboflavin/kg diet. All the ducks were raised individually for 10 weeks and they were free access to feed and water. Eggs from each group were collected and were hatched weekly. The result showed that egg hatchability of the riboflavin-deficient group dropped markedly since 2 weeks of the experiment compared with the control group ($P < 0.05$) which hatchability above 79%, and decreased further to zero after 6 weeks. Meanwhile, yolk riboflavin concentration reduced in the RD group ($P < 0.05$) compared with the control group since 2 weeks of the experiment, which is consistent with the reduction of hatchability. To investigate the mechanism of embryonic death due to maternal riboflavin deficiency, embryonic liver from the two groups were collected to find differentially expressed proteins by a proteomics analysis. The proteome results showed that two flavoproteins, acyl-CoA dehydrogenase family member 9 (ACAD9) and short-chain specific acyl-CoA dehydrogenase (ACADS), were down regulated in the RD group compared with the C group. These two flavin-dependent mitochondrial enzymes catalyze the initial rate-limiting step in the β -oxidation cycle. This result indicates that the reduction of the proteins of ACAD9 and ACADS in response to riboflavin deficiency in maternal diet could impair embryonic liver β -oxidation and lead to less energy production, resulting in the death of embryos. In conclusion, riboflavin deficiency in maternal diet of ducks could impair embryonic development via blocking liver β -oxidation.

Keywords: ducks, riboflavin, deficiency, embryonic development, proteomics

S12- 0057 Nutritive value of citrus pulp and its effect on performance in goose from 35 to 70 days of age

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Three experiments were conducted to determine the chemical composition, nutritive value of dried citrus pulp (DCP) and its effect as a feed supplement on the performance of geese. The results of Experiment 1, in which the chemical composition of DCP was examined, showed that the gross energy, dry matter, crude protein, ether extract, crude fibre, calcium, phosphorous and amino acid content of mulberry leaves was 15.08 kcal/kg, 90.75%, 6.17%, 3.51%, 15.69%, 3.69%, 0.13%, and 0.02% to 0.54%, respectively. In Experiment 2, the energetic values and digestibility of amino acids of DCP were analysed using the emptying followed by force-feeding method. The apparent metabolizable rate of energy and true digestibility of amino acid of DCP by geese were 58.61% and 49.69-81.57%, respectively. In Experiment 3, the effectiveness of DCP as a feed supplement for geese was investigated. Two hundred and ten 35-day-old male Sichuan white geese with an average initial body weight of 1767g were randomly allocated to 5 treatments, each consisting of 6 replicate pens of 6 birds per pen. Each treatment group was fed one of 5 experimental diets containing 0%, 4%, 8%, 12% and 16% DCP until 70 days of age. Geese that were fed diets containing 4% DCP exhibited greater average daily gain (ADG) ($P < 0.05$) compared to other 4 groups. Geese fed diets supplemented with 16% DCP had increased average daily feed intake (ADFI) ($P < 0.05$) and a higher feed: gain ratio (F: G). ($P < 0.05$) than those fed the diet containing 0-12% DCP. Moreover, inclusion of DCP in diet did not affect the yields of breast meat ($P < 0.05$), leg meat ($P > 0.05$), subcutaneous fat and skin ($P > 0.05$), and abdominal fat ($P > 0.05$) across 5 treatment groups. Thus, results from the current study suggest that DCP is a potentially useful feed ingredient for geese, and can be included below 12% in diet without negative effects on growth performance. This work was supported by research project 201303143, CARS-43, 2011BAD26B01 and 15401).

Keywords: dried citrus pulp, chemical composition, nutritive value, growth performance, carcass yield, geese

S13-0003 Diversified poultry production in India

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India ranked the third largest egg producer and the fourth largest meat producer in the world. But the consumption of egg and chicken meat is too low when compared to developed countries. In order to increase the per capita consumption and improve the livelihood of the farming community, turkey, duck, Japanese quail rearing can be undertaken. A data on various management practices of Japanese quail, turkey and duck were collected from 450 farmers of different agro-climatic zones of Tamil Nadu and analyzed. Housing, feeding and marketing pattern are discussed in details. Japanese quail has more resistance to common diseases of poultry and thrives well in various agro-climatic conditions. Japanese quail requires less investment and gives quick returns, higher profits and hence can be adopted by rural masses quickly. Rearing 1000 quails every week will generate the income of US \$ 12,000 per year. Turkey rearing is suitable for small and marginal farmers as the turkey can be easily reared in free range or semi-intensive system with minimal investment for management. Rearing 1000 turkeys will generate the income of US \$ 13,850 for two batches in a year. Ducks needs lesser attention and supplements their feed by foraging, insects, snails, earthworms, small fishes etc. Ducks are suitable for integrated farming systems such as duck-cum-fish farming, duck farming with rice culture. Keeping 1000 ducks will generate the income of US \$ 3,200 per year. Hence, rearing of Japanese quail, turkey and duck will enhance the livelihood of small and marginal farmers of developing countries.

Keywords: Japanese quail, turkey, duck- production

S13- 0004 Effect of stress and anti-stressors on tissue regression, corticosterone and mRNA expression of IGF-1, survivin and caspase-2 genes in hierarchial follicles of Japanese quail

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The present investigation was carried out to elucidate the effect of stress and anti-stressors on tissue regression, corticosterone and mRNA expression of IGF-1, survivin and caspase-2 genes in hierarchical follicles (F1,F2&F3) of Japanese quail. Seventy two laying Japanese quail (10 weeks) were equally divided into three groups. The former group served as control whereas birds from II and III were subjected to stress on feed withdrawal for ten days. Birds from group III were provided water ad-libitum as others along with anti-stressors (vitamin C & E @250ppm each). Each stroke sacrificed four birds from each group on day 1,2,4,6,8 and 10. Body weight, reproductive organs weight and serum extraction were exercised following standard protocol for the assessment of tissue regression and corticosterone. Hierarchical follicles were subjected to cDNA preparation which was used for gene expression by real-time PCR technique. Birds from group-II and III lost their body weight 42.68 and 35.49 percent respectively and revealed a significant ($P<0.05$) reduction in the reproductive organs weight. Compared to control, significantly ($P<0.05$) higher level of corticosterone was at any point of the experiment. The gene expression study in hierarchical follicles confirmed a significant down regulation in IGF-1. The mRNA expression of both survivin and caspase-2 revealed a significant up regulation though the former gene expression was more drastic in F2 follicle in both the treatment groups. Larger follicles in both the treatments were more sensitive to caspase-2 gene and higher fold of expression was recorded in group II. From this study it can be concluded that the degree of stress alleviation may be the cause of anti-stressors supplementation though no conclusive evidence could be drawn upon gene expression. Hence, the present observation encourages long-term care may reduce the adverse effect of stress on reproduction and restoring the body homeostasis in Japanese quail.

Keywords: stress, vitamins, expression, hormone, Japanese quail

S13– 0006 Production performance of Nandanam Japanese quail–III under Indian tropical condition

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The Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, India has released Nandanam Japanese quail I, II and III for the benefit of the farming community. Nandanam Japanese quail III has more resistance to common diseases of poultry and thrives well in various agro-climatic conditions. Nandanam Japanese quail- III requires less investment and gives quick returns, higher profits and hence can be adopted by rural masses quickly. Floor space requirement for five Nandanam Japanese quail-III is 1 sq. ft. Nandanam Japanese quail-III will consume around 500 g of feed and FCR is 2.5 to 3. Female body weight (247 g) will be always higher than male body (200 g) weight. The age at marketing is 28 to 30 days. Female Japanese quail will start lay at the end of 6th week and egg production is 200-220. Weight of Japanese quail egg is 8-14 g and day old Japanese quail chick vary from 7-11 g. A sex ratio of 1: 3 (Male: Female) will give optimum fertility rate and hatchability is 75-80 % and 65 to 70 % respectively. The total cost of production of one Japanese quail is \$ 0.32 per quail. Rearing 500 Nandanam Japanese quail III every month will generate the income of \$1711 per year. Hence, rearing of Nandanam Japanese quail-III will enhance the livelihood of small and marginal farmers.

Keywords: Nandanam Japanese quail- III, production performance, economics

S13– 0007 Production performance of Nandanam guinea fowl– I under tropical climate

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The Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University has released Nandanam Guinea fowl-I for the benefit of the farming community. Nandanam Guinea fowl-I is a hardy bird will thrive under different agro- climatic conditions. Nandanam Guinea fowl-I is being reared for egg and meat purpose. Floor space requirement for Nandanam Guinea fowl-I is 1.5 sq. ft. Body weight of Nandanam Guinea fowl-I is 1.1 kg to 1.2 kg and it will consume 3.2 kg of feed upto marketing age of 16 weeks. Nandanam Guinea fowl-I will start lay at 24 weeks of age and egg production is 150-160. An egg weight of Nandanam Guinea fowl-I is 38-40 g and weight of day old keet is 25-29 g. The sex ratio of 1:4 will give optimum fertility rate. The total cost of production of Nandanam Guinea fowl-I is \$1.62. Rearing 500 Nandanam Guinea fowl-I per batch for three batches will generate the income of \$ 1430 per year. Hence, rearing of Nandanam Guinea fowl-I will provide nutritional security as well as employment potential to family labours.

Keywords: Nandanam Guinea fowl- I, production performance, economics

S13– 0008 Production performance of Nandanam turkey under tropical climate

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Turkey rearing is seasonal and has high marketing demand every year from the month of December to January. Turkey is mostly reared under coconut trees, let loose in the fields and in some places maintained at deep litter system of management. It will thrive under different climatic conditions. TANUVAS has released Nandanam Turkey-I and II for the benefit of the farming community. Nandanam Turkey- I is suited for backyard rearing and Nandanam Turkey- II is suited for commercial farming. Floor space requirement for Nandanam Turkey is 4 sq. ft. Body weight of male and female Nandanam Turkey I is 2.5 kg and 2 kg respectively. Male and female Nandanam Turkey I will consume 10 kg and 8 kg of feed upto marketing age and FCR is 4. Body weight of male and female Nandanam Turkey- II is 3.6 kg and 2.6 kg respectively. Male and female Nandanam Turkey-II will consume 15 kg and 11 kg of feed upto marketing age and FCR is 4.25. The age at marketing is 16 weeks. Nandanam Turkey will start lay at 24 weeks of age and egg production is 100-120. An average weight of an egg is 75 g and weight of day old turkey poult is 50 g. The sex ratio of 1:3 will give optimum fertility rate. The total cost of production of Nandanam Turkey is \$ 8.85. Rearing 1500 Nandanam Turkey per year (500 turkey in one batch) will generate the income of \$ 2213 per year. Hence, rearing of Nandanam Turkey will provide nutritional security as well as employment potential to family labours.

Keywords: Nandanam Turkey-I and II, production performance, economics

S13–0009 Effect of different levels of probiotic and physical form of feed on Immune system in Japanese quail

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The aim of this study was to investigate the effect of different levels of probiotic and physical form of feed on immune system in Japanese quail. Thus, 240 Japanese quail chickens were used in a factorial experiment with completely randomized design with 6 treatments, 4 replicate and 10 chickens in each replicate for 42 days. The experimental treatments were, Mash feed without probiotic in water, Mash feed with 1% probiotic in water, Mash feed with 2% probiotic in water, Crumble feed without probiotic in water, Crumble feed with 1% probiotic in water, Crumble feed with 2% probiotic in water. The experimental diets were formulated based on national research council and UFFDA software. In order to analyzing cells blood count (CBC), one chicken from each pen was selected and the blood sample was taken from wing vein. Data were analyzed with GLM procedure using SAS 9.1. Means were compared via least square means and Tukey test ($P < 0.05$). The results showed that the experimental treatments had significant effect on lymphocytes and monocytes of Japanese quail chickens ($P < 0.05$). Chickens fed with crumble feed without probiotic in water had the highest lymphocytes and chickens receiving mash feed without probiotic in water had the highest monocytes. There were no significant differences in heterophiles and basophils of examined birds ($P > 0.05$). According to the results, it seems that protexin probiotic improved immune system of Japanese quail chickens.

Keywords: protexin probiotic, feed physical form, immune system, Japanese quail chickens.

S13- 0010 Growth performance, body measurements and slaughter characteristics as influenced by different patio strategies in Japanese quails

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Generally the chicks are removed from hatcher only when most of chicks have hatched within the period of hatch window which is almost 36 to 48 hours. A little less space in the hatcher, opening of hatcher over many time and late collection of chicks can lead to dehydrated and low quality chicks. To reduce above stated risks a concept named "Patio system" was introduced. Patio hatching is a novel idea of combined hatching and brooding. Although this system has shown a number of advantages, yet, the people are still reluctant to adopt this technology. Present study was conducted to assess the usefulness of patio system (Patio) and probably is the first of its kind on Japanese quails. A total of 900 chicks hatched from prior incubated eggs were distributed in 5 Patio strategies. In each strategy, the duration of combined hatching and brooding varied. In patio 0 conventional hatching and then shifting to brooding shed at the end of hatching was practiced. In patio I, during the last three days of incubation birds were offered feed and water with in the hatcher, while in patio III, V & VII, after shifting from setter to hatcher, chicks were brooded within the same incubator for 3, 5 and 7 days respectively. The provision of feed and water was ensured with in the hatcher. Patio strategies significantly ($P \leq 0.05$) influenced body weight, gain, and times of gain, Patio strategies also affected body, drumstick, keel, and shank length, wing spread, drumstick and shank circumference as well as thigh meat yield, liver and heart %. Regarding overall growth performance patio system III proved the best while regarding slaughter parameters and body measurements patio system V was found to be most significant. On the basis of this study, it is conclude and suggest that patio hatching can be successfully applied in the quail production sector, however future interventions through comprehensive studies may further improve its usefulness.

Keywords: growth performance, body measurements, slaughter characteristics, patio strategies, Japanese quail

S13- 0011 Sulfur amino acids requirements of male Korean native ducklings from hatch to 21- day of age

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A dose-response experiment was conducted to determine the sulfur amino acids requirements for male Korean native ducklings from hatch to 21-day of age. Three-hundred and thirty-six day-old male Korean native ducklings were used in a completely randomized design with 6 replicates per treatment and 8 ducklings per pen. The concentrations of total sulfur amino acids used in this experiment were 0.62, 0.65, 0.68, 0.71, 0.74, 0.77 and 0.80%. A wheat, corn and soybean meal-based basal diet was formulated to meet the NRC (1994) nutrient specifications, and also to achieve an ideal amino acid pattern, except sulfur amino acids. Ducklings were weighed individually and were randomly allocated to 1 of 7 dietary treatments with varying dietary sulfur amino acids concentrations based on body weight at arrival. Ducklings were offered the experimental diets on an ad libitum basis for the study, and fresh water was available at all times. Body weight and feed intake were measured weekly to calculate feed conversion ratio. One duckling per pen ($n=6$) were euthanized via cervical dislocation to measure empty body and drumsticks weights at the conclusion of experiment. Total sulfur amino acids requirements for Korean native ducklings from hatch to 21 days of age were estimated to be 0.68, 0.68, 0.65 and 0.68% for maximum body weight, daily gain, daily feed intake, and for minimum feed conversion ratio, respectively, when data were fitted to a liner and quadratic-plateau model.

Keywords: Korean native ducklings, linear-plateau model, total sulfur amino acid, quadratic-plateau model

S13-0012 The relationship of eritrosit arginase activites and urea levels in different species

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In this study arginase activites and urea levels are found in eritrosite of different species. According to the results, very close relationship is found between the enzyme activity and eritrosit hemolizat urea. Thiosemicarbazide diacetylmonoxime urea (T.D.M.U) method that used to find the urea levels checked by putting hemolizats and urea into the reaction and then urea levels after the reaction are measured to be able to understand if TDMU method is unique to urea. After the urea reaction it is observed that urea levels in the hemolizates are at the unmeasurable levels. The arginine levels in cow eritrosites are more active than sheep eritrosits. The idea of the arginase levels in the goat eritrosits are less resistant can be suggested because arginase activities in goats are at the unmeasurable levels, but in their eritrosit hemolizats measurable levels of urea are found. The arginase activity levels in chicken eritrosits are so low that is unmeasurable. In addition to that the urea levels are also at the unmeasurable levels in chicken eritrosits. This status is suggested by that arginase enzyme is not present in chicken eritrosits. The evident stokiometric relationship has been determined between urea and ornithine levels after arginine reaction in cow and sheep eritrosit.

Keywords: arginase, urea levels, chicken, eritrosit, cow, sheep

S13- 0013 Effect of dietary electrolyte balance and crude protein content on foot pad dermatitis in commercial turkeys

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Factors such as dietary electrolyte balance (EB) and crude protein (CP) content, age, and strain may affect the prevalence of foot pad dermatitis (FPD). The objective of the study was to evaluate the effect of decreasing EB (high EB (HEB) vs. low EB (LEB)) and CP (high CP (HP) vs. low CP (LP)) in two turkey strains on growth performance, litter quality and FPD in a 2x2x2 factorial block design. A total of 1920 male poults were housed in 64 pens (3 m wide x 4 m deep) littered with wood shavings at a stocking rate of 30 poults/pen. Diets were formulated isocaloric for 5 phases (0-28, 28-56, 56-84, 84-112 and 112-134 days of age) and containing per phase 290 vs. 260, 270 vs. 240, 230 vs. 200, 200 vs. 170, 170 vs. 140 g CP/kg, respectively; and EB (240 vs. 130 mEq/kg) in all phases. Free amino acids were supplemented to the diets according to breeder recommendations. LEB diets were formulated by exchange of soya bean meal by maize gluten meal, peas, potato protein, rapeseed meal and sunflower seed meal. Water and feed were provided ad libitum. Body weight, feed intake, litter moisture and FPD scores were recorded at 28, 56, 84, 112 and 134 days of age. Body weight gain was not affected by CP and FCR was significantly higher on LP than on HP diets (2.56 vs. 2.50; P=0.002). FPD score of turkeys fed on LP was lower than on HP until 84 days (P<0.001). LEB resulted in a significantly lower feed intake (420 vs. 435 g/d) and body weight gain (166 vs. 172 g/d) over the period 28 to 124 days and lower body weight (18588 vs. 19405 g) at 134 days of age compared with HEB whereas FCR was not affected. Litter was significantly drier on LEB than HEB diets (P<0.001). FPD score on LEB was significantly lower than on HEB diets (P<0.001). FPD was not affected by turkey strain. It is concluded that litter quality can be improved and FPD can be decreased in turkeys fed on diets containing lower CP and EB levels.

Keywords: foot pad dermatitis, growth performance, litter quality, protein, turkeys

S13- 0014 Six Generations of selection for higher four week body weight in Japanese quail

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Japanese quails are considered as model experimental birds with excellent nutritive value in terms of its meat and eggs. Quail production has the potential to serve as an alternative source of animal proteins in Pakistan, yet its poor growth and reproductive performance are the main bottle necks. The present study was conducted at Avian Research and training (ART) Center, University of Veterinary and Animal Sciences Lahore, Pakistan with the main objective to improve four week body weight in Japanese Quails through selective breeding. After six generations of selection, significant ($P<0.05$) improvement in body weight was observed. Day old chick weight improved significantly (8.08 to 9.65) and the same was the trend for four week body weight (149.40 to 243.50). Egg quality, hatching traits and the overall immune profile did not show any significant variation as a response to selection for higher body weight, however, a little decline in egg production was observed with the advancement in generations. The results of the present study are very promising, suggesting selective breeding to be an important tool to improve growth performance in quails without showing any major decline in reproductive performance and immune profile.

Keywords: Japanese quail, selection, body weight, egg production, egg quality, hatching traits, immune profile

S13-0015 Effect of Lactobacillus plantarum supplementation from guinea fowl origin on growth performance, gut health and immunity in host species

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Extensive use of antibiotics and chemicals for treatment and prevention of enteric disease has resulted in environmental as well as human health concern recently. In this scenario feed supplements like probiotics, prebiotic, nucleotides etc will be effective to counteract problem. Therefore current study was conducted to compare growth, humoral immunity; competitive exclusion and immune related gene expression in diet supplemented with guinea fowl gut isolated Lactobacillus plantarum (G.I.L.p.). G.I.L.p was isolated from intestine of guinea fowl, selection were based on their probiotic potency through various in vitro tests. Day old keets were weighted and randomly placed in four treatment groups each containing fifty keets. Four dietary treatments viz., T1 (basal diet+ G.I.L.p.@108cfu/gm of feed), T2 (basal diet+ G.I.L.p.@108cfu/gm of feed + mannanoligosaccharide (MOS) @ 0.1% of basal feed), T3 (multi-strain commercial probiotic @ 0.1% of basal feed) and T4 (Basal diet) were offered from 0-12 weeks. Zoo- technical parameters between 0-12 weeks were not differ significantly due to probiotics supplementation. The humeral immunity (log₂ ND-HI antibody titre) was significantly ($P<0.05$) higher in all probiotic supplemented groups as compare to control. E. coli and Salmonella count in crop, ileum and caecum was lowered ($P<0.05$) in all probiotic supplemented groups as compare to control. Relative expression of IFN-gamma and IL-6 was significantly ($P<0.01$) down regulated in all probiotic supplemented in groups as compare to control. Supplementation of G.I.L.p. @108cfu/g of feed resulted in improvement of immunity, exclusion of pathogenic bacteria as well as reduction in the expression of pro-inflammatory cytokines; this result seems to improve further more when given in combination with MOS. It was recommended that supplementation of G.I.L.p. (108cfu/g)+MOS (0.1%) used for betterment of production, immunity as well as welfare of guinea fowls.

Keywords: Guinea fowl, L. plantarum, immunity, competitive exclusion

S13– 0017 Effects of Chinese herbal medicine on growth performance, slaughter performance and blood biochemical parameters of pigeons

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Antibiotics can promote the development of livestock. However, the long-term use in livestock may pose a risk to human health. Chinese herbal medicine is a kind of material which has the function of both nutrition and medicine. It can improve the performance and immune function of livestock with no drug resistance and relatively less toxicity. This study was conducted to compare the effects of Chinese herbal medicine and antibiotics on growth performance, slaughter performance and blood biochemical parameters of pigeons. Seventy-two pairs (male & female) of breeding pigeons were selected and randomly divided into 3 groups: control group, antibiotic group (41.4 mg/kg doxycycline and 10 mg/kg florfenicol) and Chinese herbal medicine group (0.1% a mix including honeysuckle, lanceolata, astragalus, et.al). There were 4 replicates per group with 6 pairs of breeding pigeons and twelve squabs per replicate. The weight of squabs and feed intake were recorded weekly during the experiment. On day 28, two pigeons per replicate were randomly slaughtered to determine blood biochemical parameters and slaughter performance. Chinese herbal medicine increased the feed intake ($P < 0.05$). Antibiotics increased the half eviscerated carcass ratio and eviscerated carcass ratio ($P < 0.05$). The wing muscle ratio and the leg muscle ratio of Chinese herbal medicine group was higher than that of antibiotic group ($P < 0.05$). Both Chinese herbal medicine and antibiotics increased the low-density lipoprotein cholesterol (LDL-C) ($P < 0.05$). Compared with antibiotics, Chinese herbal medicine could improve growth performance and slaughter performance.

Keywords: Chinese herbal medicine, antibiotics, pigeon, growth performance, slaughter performance

S13– 0018 Efficacy of a live Escherichia coli vaccine for protection of turkeys against homologous and heterologous field strains infection

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This work aimed to evaluate the efficacy of a commercial live attenuated *E. coli* vaccine in turkeys against experimental homologous and heterologous colibacillosis infection. Sixty specific antibody negative one-day old turkeys were vaccinated twice by aerosol spray with the vaccine under test. Another thirty turkeys were kept as non-vaccinated control. Birds were challenged against homologous O78 and heterologous O1 and O2 pathogenic field strains of *E. coli* using 107 CFU/0.2 ml/ turkey at 5th weeks post vaccination. On 8th day post challenge, mortality was recorded and surviving turkeys were euthanized and examined for the recovery of *E. coli* organism from heart blood, liver, spleen and bone marrow samples. Antibody response in sera of vaccinated and non-vaccinated turkeys was assessed weekly by Micro-agglutination test. The protection rate of vaccinated turkeys post challenge with homologous (O78) was 96.7%, while birds were unable to withstand the heterologous challenge. *E. coli* was recovered from vaccinated challenged turkeys at ratio ranged from 13.3%-20% from the bone marrow, liver, spleen and heart blood post homologous challenge while these ratios were ranged from 53.3%-66.7% post heterologous challenge. There was a marked increase of *E. coli* antibody titers against strain O78 in sera of vaccinated birds in comparison with the unvaccinated ones. Live-attenuated *E. coli* vaccine was protective against homologous challenge with O78 strain but could not protect turkeys against heterologous challenge.

Keywords: turkey, *E. coli*, vaccine

S13-0019 Effect of light quality and barn enrichment on the prevalence of injurious pecking in intact-beak tom turkeys

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In Germany, beak trimming is common practice in commercial turkey farms but it will be banned. Keeping intact-beak turkeys may cause more undesirable behaviour like injurious pecking. In turkeys, lighting influences behavioural traits and performance but knowledge regarding the effects of light quality or emission spectrum on the behaviour and well-being is limited. The aim of this study was to evaluate the influence of light quality and barn enrichment on the prevalence of injurious pecking in turkeys. In each of two trials 600 male day-old B.U. T. 6 turkeys were allocated to six pens (each 36 m², 2.8 toms/m²). The pens were illuminated by two tubular fluorescent lamps (each 58 W) with light temperature of either 3000 K or 6500 K. Each pen consisted of two parts (both enriched with a wheat grain feeder) that were connected with a passage which was equipped with two antennas. All birds were marked with two leg transponders and kept for 20 weeks under otherwise common conditions. Video recordings were used to evaluate pecking behaviour and the use of the enrichment. Performance and health data were determined. The effect of light spectrum and luminance intensity (20 lx vs. > 20 lx) on the use of the feeders was tested using GLM. The prevalence of injurious pecking in dependence of the use of feeders and light quality was evaluated by a generalized linear mixed Poisson model. In both trials injurious pecking started in the first week of life and occurred more frequently ($P < 0.0001$) when the pens were illuminated with warm white light (3000 K). Wheat grain feeders were used more at 20 lx ($P = 0.0213$). They were used between 1 s and 21.4 min. The prevalence of injurious pecking decreased with increasing use of feeders ($P < 0.0001$). Mortality was significantly influenced by trial ($P = 0.04$). The study indicates that light quality and barn enrichment seem to have an important influence on the prevalence of injurious pecking but further studies are necessary.

Keywords: tom turkeys, injurious pecking, light quality, enrichment, intact-beak

S13-0023 The effects of using garlic extract in quail hatching egg disinfection on incubator results and performance

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This experiment was carried out to determine the effects of using garlic extract (*Allium sativum*) as an alternative to formaldehyde disinfection of hatching eggs on hatchability parameters and growth performance of quails. In the experiment four groups were formed (two different levels (2.5% and 5.0%) of garlic extract (garlic-1, garlic-2), formaldehyde and a disinfection process without any (negative control)) and each treatment group consisted of 240 hatching eggs. The middle term, the last term, pipped mortality and discarded chick rate were found statistically insignificant between treatments ($P > 0.05$). The highest early embryonic mortality rate was found 9.99% in formaldehyde treatment, the lowest was found in garlic-2 treatment (2.68%) and differences between treatments were obtained statistically significant ($P < 0.05$). Hatchability of fertile eggs, was the lowest in the formaldehyde group (83.78%) and this was followed by control (85.20%), garlic-1 (87.11%) and garlic-2 (88.72%), respectively. The highest fifth week body weight was obtained from garlic-2 (304.1 g), and this was followed by control (294.13 g), formaldehyde (290.56 g) and garlic-1 (288.44 g), respectively and differences between treatments was found statistically significant ($P < 0.05$). Feed intake and feed conversion rate have not changed according to treatments ($P > 0.05$). Feed efficiency ratio deteriorated with increasing age. The results, obtained from hatchability parameters and fattening performance of chick showed that garlic extract which is a natural product can be used as an alternative source to formaldehyde for hatching eggs fumigation.

Keywords: quail, hatching eggs, disinfection, garlic extract

S13-0024 Ostrich's eggs: the productivity, quality and hatchability

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Almost from three decades, there has been worldwide farming of ratite family especially ostriches for feather, meat, egg, skin and oil production. Ostriches lay the largest eggs in the world; their eggs are very hard and tough, it could be used as table eggs or hatching eggs or in decoration and adornment. Both environment and genetic factors affecting the ostrich egg production, so the egg production varies from 25 to 120 eggs per season. According to several studies, the variability of external and internal egg quality values may be attributed to weather, location of the farm, nutrition and physiological factors. Genetic impact also plays an important role in this concern. Breeder factors that affect hatchability include strain, health, nutrition and age of the flock, egg size, weight and quality, egg storage duration and conditions. Globally, there is no doubt that the ostrich production should take more interest due to their several and unique products not only for human food but also for industrial use (leather, oil, feather and medical industries). This review aims at take an overview about the productivity, quality and hatching traits of ostrich's eggs.

Keywords: ostrich, egg production, quality traits, hatching traits

S13- 0025 Different levels of autolyzed yeast with enzymatically hydrolyzed RNA as a nucleotide source in turkey starter diets

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This study evaluated the effects of dietary supplementation of 3 levels of autolyzed yeast (with enzymatically hydrolyzed RNA) as a nucleotide source on turkey performance. For this, 256 one-day-old Nicolas turkeys poults were distributed in a completely randomized block design (battery pens), with 4 treatments and 8 blocks, being 8 cages of 8 poults each (0.19 m²/bird). The treatments were: T1- Control diet (no additive supplemented); T2- Diet with nucleotide source (1 kg/MT), T3- Diet with nucleotide source (3 kg/MT); T4- Diet with nucleotide source (5 kg/MT). The source of nucleotide [YNU] was from *Saccharomyces cerevisiae* yeast product named Hilyses®, included in diets from 0-21 d. Study criteria included body weight gain (BWG, kg), feed intake (FI, kg), feed/gain ratio (FGR), and mortality rate (%) at 21 d. Duodenum and jejunum samples were collected at 21 d for measurement of villus height (V, µm), crypt depth (C, µm), relationship between villus height and crypt depth (V:C ratio), and mucosal thickness (MT, µm). The data were analyzed using the SAS LSD test ($P \leq 0.05$) to separate means when ANOVA F values are significant ($P \leq 0.05$). The birds supplemented with YNU at 1 kg/MT had best FGR (-6.85 and -5.80%) when compared to Control and YNU at 5 kg/MT ($P < 0.05$), respectively. The treatments had no effect ($P > 0.05$) on FI, BWG, or mortality rate at 21 d. Although there were no statistical differences ($P > 0.05$) between treatments, histomorphometry parameters for each YNU inclusion level (1, 3 and 5 kg/MT, respectively) were numerically improved in duodenum (V +3.8, 8 and 8%; V:C +12.3, 16 and 8.3%; MT +1.8, 5.1 and 7%) and jejunum (V +14.2, 12 and 23.4%; V:C +28.3, 13.6 and 39%; MT +9, 8.3 and 16%) versus Control. In summary, this study demonstrated that turkey diets supplemented with 1 kg/MT of YNU significantly improved feed/gain ratio from 0-21 d compared to control diets.

Keywords: intestinal integrity, performance, *Saccharomyces cerevisiae*

S13-0026 Effect of inclusion of Foxtail millet sprout grown under hydroponic system on growth performance of Nandanam Japanese quail

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A study was conducted to evaluate the effect of inclusion of foxtail millet sprout grown under hydroponic system on growth performance of Nandanam Japanese quail at University Research Farm, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India. The proximate analysis of foxtail millet sprout revealed that the content of moisture, crude protein, crude fiber, ether extract, nitrogen free extract and total ash (dry matter basis) were 75.08, 14.69, 12.11, 5.38, 64.23 and 3.59 per cent respectively. A total of 1254 Nandanam Japanese quail of day old chicks were housed in cage system of management and were randomly divided into three treatment groups with 4 replicates of 132 Nandanam Japanese quail chicks each. An experimental feeds were prepared by including foxtail millet sprout zero gram with 30 gram concentrate (control, T1), foxtail millet sprout 30 gram with 15 gram concentrate (T2) and foxtail millet sprout 50 gram with 5 gram concentrate (T3) were feed to Nandanam Japanese quail for a period of five weeks under standard managerial conditions. The parameters like body weight, weight gain, feed conversion ratio (FCR), livability were recorded. Highly significant results were observed ($P < 0.01$) in body weight and weight gain. Higher body weight was noticed in T2 followed by T3. The Feed consumption, feed efficiency and livability did not show any significant difference. It is concluded that the inclusion of foxtail millet sprout grown under hydroponic system enhanced growth performance of Nandanam Japanese quail with better return and reduce cost of production.

Keywords: Nandanam Japanese quail, foxtail millet sprout, Hydroponic, growth performance

S13-0027 The electrolyte supplementation of commercial Pekin ducks during short periods of high ambient temperature

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The electrolyte supplementation of commercial Pekin ducks during short periods of high ambient temperature. J.A. Downing School of Life and Environmental Science, Faculty of Veterinary Science, University of Sydney, New South Wales, AUSTRALIA. The objective of the study was to assess the effects of electrolyte supplementation on the performance of commercial Cherry Valley Pekin ducks reared as mixed sex groups when exposed to short periods of high ambient temperature during week 5 and week 6 of age. Control birds (C) were provided with tap water only while treatment birds received electrolyte solution (E) consisting of 126 g NaCl, 214 g Na H₂CO₃ and 111 g KCl dissolved in 100 L of tap water. The treatments were applied on days 30-32 and days 40-42 when the temperature was raised to 32°C from 08:00 to 17:00 and then reduced to 24°C from 17:00 to 08:00. Over days 33-39 temperature was maintained at 20°C. Treatment had a significant effect on daily live weight gain (LWG) but this changed with time as the treatment x day interaction was significant ($P < 0.001$). For treatments the gain per day was significantly different in all periods ($P < 0.05$). During the first heat period (days 30-32) the birds given E had superior daily LWG (76 ± 2 g) compared to C birds (57 ± 2 g) ($P < 0.05$). Over days 33-39 the treatments has similar LWG. During the second heat period (days 40-42) the control birds had the poorer daily LWG (33 ± 1 g) compared to those supplied with E (60 ± 2 g). Ducks benefit from supplementing water with additional electrolytes during periods of high temperature. This is a practical option for producers as the application can be targeted to specific periods of high temperature or when heat-wave conditions are anticipated.

Keywords: Peking ducks, heat stress, electrolytes

S13- 0028 Differential expression of cytokines in spleenocyte of guinea fowl and chicken after In Vitro exposure to Salmonella enteric serovar Enteritidis

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The in-depth knowledge of mechanism of disease resistance is important to unravel the host-pathogen interactions for the manipulation of immune responses to develop effective control strategies against poultry diseases. Since, guinea fowl has the unique characteristics related to resistance to the common diseases occurring in chicken. Therefore, the present study was aimed to study the Differential expression analysis of different cytokines in in vitro cultured spleenocytes of guinea fowl and chicken. Screening of birds for circulating antibodies against Salmonella species was done. Purity of Salmonella enterica serovar Enteritidis (SE) culture was checked. Gene specific standard curve was generated using qRT-PCR to estimate the primer binding efficiency. In vitro mRNA expression kinetics of different cytokine genes, in SE induced and uninduced spleenocytes of GF and CK at different p.i. time, was estimated in terms of LS mean adjusted 40-CT values and fold change. All analyses were performed using the GLM procedure of JMP statistical program package. Expression of Pro-inflammatory cytokines (IL-1 β , IL-6 and TNF- α) was very high in guinea fowl in comparison to broiler particularly at 1 & 12 hrs p.i. In contrast, Guinea fowl spleenocytes expressed anti-inflammatory cytokines (TGF- β 4) at lower level than broiler, particularly at 12 hrs p.i., which may be the cause of the higher expression of pro-inflammatory cytokines at 12 hrs p.i. in GF. Expression of the IL-10 (Th2) cytokine was exceptionally higher in GF than broiler. In vitro Pathogen induction studies revealed that guinea fowl was more responsive than broiler chicken in terms of expression of immune molecules. The unique response of guinea fowl was in contrast to the broiler which had the history of intense genetic selection for the increase growth. Finally, the results of the present investigation are encouraging to establish the guinea fowl as a model for the disease resistance studies in poultry species.

Keywords: guinea fowl, disease resistance, cytokines, differential expression, spleenocytes

S13-0029 Growth modeling of Japanese quail with different non- linear models

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The objective of this study was to estimate the growth curve parameters for live body weight of Japanese quail comparing both sexes through applying different non- linear models. The data were collected from 1260 growing Japanese quails came from 126 dams and 63 sires weighed weekly from hatch to Thirty-five days of age. Four different non-linear models, namely Gompertz, Richards, Logistic and Weibull, were used to define the growth curves of the birds comparing males to females. Analyses were performed via SAS 9.2 software. R2 values for the Gompertz, Logistic, Richards and Weibull models were 0.997, 0.995, 0.982 and 0.982 respectively for the females. The same values for males were 0.993, 0.997, 0.980 and 0.979, respectively. The Gompertz model had the highest coefficient of determination (R2) value for females, while Richards model was the best for males. Logistic and Weibull models were inferior in (R2) for both sexes. Both Gompertz and Richards models had similar MSE (Mean Square Error), SD (Standard Deviation) and AIC (Akaike's Information Criterion) values which were less than those for Logistic and Weibull models. Therefore, based on goodness of fit criteria; R2, MSE, SD and AIC values, Gompertz model, for females, and Richards model, for males, described live body weight data of the Japanese quail very well. Both of them surpassed Logistic and Weibull models and could be suggested for describing the pooled data without lack of goodness of fit.

Keywords: Japanese quail, body weight, growth curve, Gompertz, Richards

S13- 0030 Past history, present scenario and projected profiles of guinea fowl production in Bangladesh

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The paper was aimed to focus the past history, present status and projected profiles of guinea fowls production in Bangladesh. Among the poultry species available in Bangladesh, guinea fowl was very common in past and rural farmers kept the birds along with other indigenous chicken, ducks, geese or pigeons. Because of some unique features such as premier meat quality, higher egg production, better scavenging capability, good resistance to common poultry diseases and better adaptability to environment the 'helmeted guinea' always become the 'choice' of native poultry keepers. Presently, the birds are facing extinct elsewhere in Bangladesh, resulting remarkable decreased of their population. Currently, the species is seldom observed at farmer's homesteads. In this review, attempts are made to explain the reasons behind why the population of guinea fowls noticeably declined within the span of last 2-3 decades; focuses are also given to understand the significance of production, conservation and extension of the birds and finally strategic planning are emphasized to re-establish the species again in native poultry stocks.

Keywords: Guinea fowl, Bangladesh, native poultry keepers

S13-0031 The study of extruded full fat soybean (ShaySoy™) inclusion in breeder ostrich performance

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The aim of this study was to evaluate the effects of different levels of wet extruded full fat soybean (ShaySoy™) in diets on performance of breeder ostrich. For this purpose 45 breeder ostriches were assigned to three treatments on a completely randomized design and four replicates in open shaded paddock with 2 males and 3 female birds in each, for a 60 day experimental period. Treatments included 1. Control diet based on corn/soybean meal and oil (CSO) 2. Diet containing 7% extruded full fat soybeans (EFFSB7) 3. Diet containing 15% extruded full fat soybeans (EFFSB15). The effects of treatments on ostrich breeder performance, reproduction traits and hatchability were determined. Results showed that inclusion of EFFSB in diet had no effect on egg production in overall experimental period ($P>0.05$). In EFFSB diets, the average egg weight increased significantly ($P<0.05$) compared to the control group, but egg mass was not affected by treatments ($P>0.05$). The effects of different treatments on settable eggs were not significant ($P>0.05$), in seven week of experiment the ratio of fertile to settable eggs were affected by treatments ($P<0.05$) but there was no significant differences in the overall experimental period. The number of hatched eggs per settable eggs were not affected by treatments ($P>0.05$) but in this research the increasing egg production caused more number of hatched chicks and this lead to economical profit. In conclusion, based on the results of this research, inclusion of EFFSB (ShaySoy™) in level of 7 and 15 percent in ostrich breeder diet had positive effects in reproduction traits and economical profits

Keywords: ostrich, exdtruded full fat soybean, performance

S13-0032 The effect of white oyster mushroom (*Pleurotusostreatus*) adding on the quality of unproductive quail (*Coturnixcoturnix Japonica*) abon (Shredded Meat)

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The research is the effect of white oyster mushroom (*Pleurotusostreatus*) adding on the quality of Abon from unproductive quail. This study aims to utilize quail meat into a preferred food and cheap price. Unproductive quail is a quail no longer in production and are generally 2.5 years old and a meat is tough. To increase the value of unproductive quail (*Coturnixcoturnix Japonica*) we can process it into another products that high nutritional such as Abon. Abon known as typical Indonesian food made from meat cutlet and its price is expensive. To suppress the price of Abon in its manufacture can be added with a mixture of vegetable such as white oyster mushroom (*Pleurotusostreatus*) that taste like meat. In this study was used of 4000 g of unproductive quails meat and 600 g of white oyster mushroom (*Pleurotusostreatus*). The method use in this research is an experiment with Block Randomized Design (BRD) which consist of five treatments and four replications. The treatments are the adding of white oyster mushroom as much as 0 % (A), 10 % (B), 20 % (C) dan 30 % (D). The variable was observed the content of moisture, protein, fat and the texture of unproductive quails Abon. The result of this research indicated that the adding of white oyster mushroom increased moisture and decreased a protein significantly ($P < 0.01$) but didn't affected of fat and texture of unproductive quails Abon. The adding of white oyster mushroom up to 30 % was permitted to produce culled quails Abon.

Keywords: abon, unproductive quails, white oyster mushroom, protein, texture

S13- 0034 Improving Malondialdehyde (MDA) and hematologies value using fruit noni (*morinda citrifolia*) on quail

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The purpose of this study was aimed to determine the effect of noni fruit powder in ration on Malondialdehyde (MDA) and hematologies value laying quail phase layer. The research was held from February to May 2015 at Faculty of Animal Husbandry, Universitas Padjadjaran. One hundred quails, aged four weeks were allocated in a Completely Randomized Design (CRD) with four treatment groups as T1 (control - 0.00%), T2 (0.25%), T3 (0.50%), and T4 (0.75%), with five quails per treatment, were replicates four times. The blood samples were randomly collected from twenty quails per replication, at the end of experimental. The parameters observed were MDA, erythrocyte, hemoglobin and hematocrit levels. The results revealed that dietary inclusion of Noni Fruit Powder at all levels were not significantly ($p < 0.05$) decreased the MDA value and increased the hematologies value of quails when compared to the control group (T1). Even though has reduction of MDA in T2 ($0.341 \pm 0.04 \mu\text{g}/\text{mg}$) and T3 ($0.340 \pm 0.04 \mu\text{g}/\text{mg}$) in blood level respectively when compared to control and other groups. Further, the hematologies value, the erythrocyte, hemoglobin, and hematocrit even there were no significance, but there were increasing trends: the erythrocyte from 3.31 to $3.55 \times 10^6/\text{mm}^3$, hemoglobin from 9.64 to 11.84% and hematocrit 34.4 to 36.6% . The conclusions are using Noni Fruit Powder in ration can maintained the MDA value, and the blood hematologies of laying quail in the normal range.

Keywords: noni fruit powder, quail, erythrocyte, hemoglobin, hematocrit

S13-0035 Effects of essential oil mixture (orange peel, bay leaf, thyme, and eucalyptus) supplementation to basal diet on fattening performance, carcass characteristics, blood parameters, antioxidant status of tissues and meat quality in Japanese quails exposed to low ambient temperature

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The effects of essential oil mixture (orange peel, bay leaf, thyme, and eucalyptus) supplementation to basal diet on fattening performance, carcass characteristics, survival rate, blood parameters, some antioxidant parameters of tissues and fatty acids structures of breast meat among Japanese quails (*Coturnix coturnix Japonica*) exposed to low ambient temperature (6-8°C) were investigated in this study. Totally 90 15-day-old quail chicks were divided into 3 groups with 3 repetitions and each repetition involved 10 quails. The quails were fed with basal diet (control) and basal diet in which mixture of 50 and 100 ppm essential oil (orange peel, bay leaf, thyme, and eucalyptus) (MEO) was supplemented into basal diet. In the study, a statistical difference could not be determined between groups in terms of live weight, live weight gain, feed intake, feed conversion ratio, mortality rate, and carcass characteristics ($P>0.05$). Mixture of essential oil supplemented into basal diet in quails raising in low ambient temperature considerably decreased serum glucose, triglyceride, total cholesterol, uric acid, and total protein levels compared to the control group ($P<0.05$). It was found that while malondialdehyde (MDA) ($P<0.001$) level in liver and heart tissues was the lowest in the group in which mixture of essential oil of 50 ppm was added, glutathione peroxidase (GSH-Px) ($P<0.01$) enzyme activity was the highest in the group in which mixture of essential oil 100 ppm was added. Levels of poly-unsaturated fatty acids (PUFA, omega 3, omega-6/omega3) were higher in groups supplemented with mixture of essential oil compared to the control group ($P<0.05$). Consequently, the supplementation of mixture of essential oil (MEO) into basal diets of quails decreased negative effects of low ambient temperature. We are of opinion that this mixture can be considered as a potential natural feed supplement.

Keywords: quail, oil mixture, fattening performance, blood parameters, oxidative stress, meat quality

S13-0036 The effects of dietary royal jelly, bee pollen and Ronozyme on oxidative stress of Japanese quails (*Coturnix coturnix japonica*) reared under different stocking densities

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The objective of this study was to investigate the effects of supplementation of royal jelly (ROJ), bee pollen (BEP) and Ronozyme (RON) to the basal diet on oxidative stress and antioxidant system of Japanese quails under high stocking density (HSD). Three hundred and fifty two quails were divided into 6 groups each with 4 replicates consisting of 8 quails in control group and 16 quails in the other groups. Experimental groups were arranged as control (160 cm²/quail without supplementation), HSD (80 cm²/quail without supplementation), ROJ1 (80 cm²/quail, 250 mg/kg ROJ), ROJ2 (80 cm²/quail, 500 mg/kg ROJ), BEP (80 cm²/quail, 1g/kg BEP), RON (80 cm²/quail, 1 g/kg RON). At 42 days, quails were slaughtered. The malondialdehyde (MDA) levels in the HSD group were significantly higher than those of control, ROJ, BEP, RON groups in all tissues. Plasma MDA levels were observed decrease in specially 500 mg/kg ROJ, BEP-supplemented quails compared with the HSD group ($P<0.05$). The glutathione (GSH) levels, catalase (CAT) activities in the HSD group were significantly lower than those of the control in all tissues. GSH level, CAT activity were observed increase in especially 500 mg/kg ROJ and BEP-supplemented quails compared with the HSD group in blood, liver, while they were observed increase in ROJ, BEP, RON-supplemented quails in muscle tissue. The protein level increased in the HSD group in liver tissue, while it decreased in muscle tissue ($P<0.05$). The protein level was observed increase in ROJ, BEP and RON-supplemented quails compared with the HSD group in muscle tissue. But the protein level didn't change in liver tissue in 250 mg/kg ROJ and RON-supplemented quails. It was concluded that the increase in stocking density caused oxidative stress in animals, which resulted in significant changes in antioxidant parameters. The present study suggests that especially BEP supplementation to diet had potential protective activity on oxidative stress of quails reared under HSD.

Keywords: stocking density, Japanese quails, malondialdehyde, royal jelly, bee pollen, ronozyme

S13-0037 Effects of milk thistle seed supplementation to high-calorie basal diets of quails on oxidative stress parameters

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The purpose of this study was to examine the changes to occur in oxidative stress parameters of laying quails fed with high calorie diets and effect of milk thistle seed on these changes. A total of 75 45-day-old quails including 60 females and 15 males were used in the study. The quails were divided into 5 groups with three repetitions. 4 females and 1 male were used in each repetition. The groups of the study were arranged as following; control group (C) consuming corn- soybean based basal diet, oil group (SFO) in which 5% sun flower oil was added into basal diet, oil + milk thistle group (SFO+MT) in which 5% sun flower oil+1% milk thistle seed was added into basal diet, syrup group (CS) in which 10% corn syrup was added into basal diet, and syrup+milk thistle group (CS+MT) in which 10% corn syrup + 1% milk thistle was added into basal diet. Blood, liver, kidney, and heart samples were taken during 6 animals from each experimental group were slaughtered by using decapitation method at the end of the 60 days. MDA values of blood, liver and heart tissues were similar among the groups of study ($P>0.05$). MDA level of kidney significantly increased in CS group, however addition of milk thistle seed into diet was reduced MDA level of kidney in CS+MT group ($P<0.01$). MDA levels of SFO groups slightly increased but not significant ($P>0.05$). GSH- Px, CAT activities and GSH levels of kidney were higher in control group ($P<0.01$). Addition of MT into diet significantly reduced GSH levels and GSH-Px activities of kidney ($P<0.01$). These diets caused changes in antioxidants in blood and tissues in the birds. Particularly, in quails feeding with corn syrup significantly increased lipid peroxidation in kidney. The use of both CS and MT enabled MDA levels to become normal in kidney. Supplementation of milk thistle seed into corn syrup groups may protect kidney against free radical damage.

Keywords: quail, sun flower oil, corn syrup, milk thistle, oxidative stress parameters

S14- 0001 Effect of supplementation of unconventional feed source, Azola on the production performance of Indian desi-chicken

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Family poultry production is characterized by low-input low-cost technology. Feed cost is the major input for any type of farming system. Azola contain high amount of protein and other quality nutrients. An experiment was conducted to study the effect of supplementation of unconventional feed source, Azola on the production performance of Indian desi-chicken at the Poultry Research Station, Tamil Nadu, India. A total of 270 Aseel chicks of one month old from same hatch were utilized for this study. Experimental birds were housed in deep litter system of management and were randomly divided into three treatment groups with three replicates of 30 Aseel chicks each. Experimental feed were prepared by supplementing Azola at 0 g (control, T1), 50 g (T2) and 100 g (T3) level in the feed and fed adlibitum upto 60 days under standard managemental conditions. The parameters such as body weight, feed consumption and livability were recorded and feed efficiency was worked out and statistical analysis was done by using randomized block design. At the end of 12th week birds were slaughtered and giblets yield, New-york dressed weight and ready-to-cook weight were recorded. Birds in treatment group T3 recorded significantly higher ($P<0.05$) body weight than other treated group. Non-significant differences were observed on cumulative feed consumption and livability of different treatment groups. Birds that received 100 g of azola recoded higher feed efficiency ratio when compared to other groups. Birds treated with 100 g of azola showed significantly ($P<0.05$) higher value of New York dressed weight and ready-to-cook weight. It is concluded that supplementation of azola in Aseel feed resulted in better body weight, feed efficiency and carcass characteristics.

Keywords: Indian native chicken (Aseel), azola, production performance, slaughter studies

S14-0002 Characterisation of indigenous chicken production systems in the Sudano-sahelian zone of cameroon

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From January to September 2010, studies were undertaken in rural, peri-urban and urban areas of the Sudano-sahelian agro-ecological zone of Cameroon to characterize the production system of indigenous chicken populations. The study was also aimed at generating information on village based indigenous chicken utilization, management practices, opportunities and challenges. Thirteen villages were investigated in the Far-north and North regions using a structured questionnaire. The study of Sudano-sahelian local chicken production system revealed that women are responsible for chicken rearing in households (72.5%), the level of education was variable, with 45.1% sampled farmers who have never gone to school, and only 3.9% having post secondary school of education. Auto-consumption of chicken was the general and major objective of chicken breeding in the area (23.5%). Goat, cattle, sheep and small birds were associated to chickens in the household by the proportions of 32.21, 28.11, 10.70, and 17.79% respectively. The majority of farmers (76.5%) supplement their chickens and share their house (60.8%) with their animals. Health care was negligible, since no vaccination (70.6%) and no medical treatment (54.9%) was given to animals. Diseases and predators are the main challenges (15.7%) in the local chicken production system in the Sudano-sahelian agro-ecological zone of Cameroon. Majority of interviewed chicken owners showed a great interest to boost up existing village chicken production in the area. Efforts should be made to improve the production and productivity of village chicken in a sustainable way and to shift from the extensive system to semi-intensive husbandry, with emphasis on disciplinary support of services like: health, husbandry, research, extension training and credit intervention.

Keywords: extension, foraging, genotype, local, rural scavenging, village

S14-0003 Productive and reproductive performance of native breeder chicken intensively reared in western Tamil Nadu

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A study was carried out to investigate the native breeder (Aseel) chicken in Western Tamil Nadu with respect to productive and reproductive performance under intensive management system. A total of 45 farms, 15 farms in each Western district include Coimbatore, Erode and Tirupur were selected by simple random sampling through a semi-structured interview schedule. The farms were classified into small (Upto 1500), medium (1501 - 3000) and large (above 3000) according to the farm size, with the average capacity of 600, 2271 and 5027 birds respectively. The mean age and body weight of native breeder male and female at the time of initial breeding were 191.72 ± 4.17 days and 2.57 ± 0.07 kg and 170.96 ± 1.92 days and 1.83 ± 0.05 kg respectively. The average feed consumption during brooder (Upto 14 weeks) and grower (15 - 24 weeks) stages of native breeder chicken were 4.40 ± 0.11 and 5.18 ± 0.18 kg. The daily feed consumption of male and female parent breeder (>25 weeks) was 124.27 ± 2.34 and 117.59 ± 1.95 gram per day per bird respectively. Flock mating was practiced by the majority (75.56 per cent) of the farmers with the sex ratio of 1:8 and the mean semen volume produced by male parent kept in cages was 0.54 ± 0.05 ml. The amount of feed intake to produce a dozen egg and one kg egg mass in native breeder chicken was 5.44 ± 0.16 and 9.10 ± 0.32 kg respectively. The average egg weight and annual egg production of native breeder female parent in the selected district was 46.51 ± 0.91 gm and 101.83 ± 3.21 eggs per annum. The average number of settable egg was 97.65 ± 4.62 and hatchability rate per native breeder was 73.16 ± 1.05 per cent in selected districts of Tamil Nadu.

Keywords: Aseel - productive and reproductive parameters

S14- 0004 Adoption and constrains faced by the small poultry farmers under Indian tropical conditions

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A study was conducted to understand the adoption and constraints perceived by the small poultry farmers of Tamil Nadu, India. Data from about 960 small poultry farmers from different agro-climatic conditions of Tamil Nadu was collected and analyzed. Higher income, low input technology, less incidence of disease and thrive well in summer and winter season was over all reason for adopting improved variety of desi-chicken. The constraint as perceived by the small poultry owners was recorded. Quality and reliability of chick and feed was the major constraints which were reported by all respondents. None availability of grown chick for the farmers to get maximum profit and avoid loss due to chick mortality. Lack of veterinary services, non-availability of vaccine and medicine, high cost of chicks and feed, inadequate knowledge about scientific rearing of birds and transportation facilities as reported as constrains. In order to overcome the above constrains, train the farmers on scientific rearing of birds is very essential.

Keywords: family poultry -constrains- training

S14- 0007 Evaluation of the dietary protein to energy ratio on meat production performance and carcass characteristics of Iranian native chickens

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Today's demand for native rearing and their products is growing. So a trial was conducted to determine the effect of different dietary protein to energy ratio on performance and carcass characteristics of Iranian native chickens from 1 to 63 d of age. Five experimental diets were formulated to have 5 ratio of ME to CP, respectively in each phase: 159(diet A), 149 (diet B), 139(diet C, as a control diet agree with NRC), 129(diet D), and 119(diet E) in starter phase (1 to 21 d); 180,170,160,150, and 140 in grower phase (22 to 42 d) and 197,187,177,167, and 157 in the finisher phase (43 to 63 d). A total of 500 one-day-old native chickens in a completely randomized design were randomly divided into 25 experimental pens, 20 chickens in each pen, and each diet was offered to 5 replicates at random. The results showed that average daily gain and body weight (BW) have affected by ME/CP ratio during starter, grower, finisher, and overall experimental periods ($P < 0.05$). The highest (1026 g) and the less (943 g) BW at 63 d age was belonged to diet E with the lowest ME/CP ratio and diet B with 10 unit ME/CP ratio more than control diet, respectively. Feed intake (FI) was affected just in starter period and feed conversion ratio (FCR) was affected in starter and grower periods, whereas both of them (FI & FCR) were not affected by the treatments in overall experimental periods. Protein conversion ratio increased significantly ($P < 0.001$) with high-CP diet (0.53 for diet E vs. 0.43 for diet A), whereas energy conversion ratio (ECR) was not affected by treatments in overall experimental periods, but for starter period ECR decreased by dietary CP increasing (5.99 vs. 5.50, $P < 0.05$). However, carcass yield, breast meat yield, thigh yield, abdominal fat, and alimentary tract weights were not affected by the treatments. In conclusion, dietary ME/CP ratio of 119, 170 and 177 are recommended for starter, grower and finisher periods, respectively for Iranian native chickens.

Keywords: native hen, broiler chick, energy: protein ratio, growth performance, nutrient utilization

S14-0008 Benefits of keeping native chickens and their farming systems in Indonesia

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Native chickens are commonly raised in many areas of Indonesia and play a major role in food production, often providing the main source of dietary animal protein for many people. They are often called “non-breed chickens” — (“or (“buras”) to differentiate local chickens from modern commercialized chicken breeds. There are at least 34 kinds of native chickens in Indonesia. Some of the more common native chickens, namely Ayunai, Balenggek, Banten, Bangkok, Burgo, Bekisar, Cangehgar, Cemani, Ciparage, Gaok, Jepun, Kampung, Kasintu, Kedu (Black and White Kedu), Pelung, Lamba, Maleo, Melayu, Merawang, Nagrak, Nunukan, Nusa Penida, Olgan, Rintit or Walik, Sedayu, Sentul, Siem, Sumatera, Tolaki, Tukung, Wareng, Sabu, and Semau. Some of them are used for non-food purposes. Indonesia with its population is over 258.71 million people in 2016 has an annual level of protein consumption from poultry meat of 12.97 kg/capita/year and poultry eggs exceeded 190 eggs/capita/year in 2015. Native chickens alone accounted for about 10% of Indonesia’s total meat consumption compared to broiler (55%), beef (19%), pork (8%), goat (7%), and others (1%) with its per capita meat consumption from livestock is still lower compared to many countries. There are three types of farming systems are used to raise native chickens in Indonesia. First, the extensive traditional system, and farmers usually reared native chickens ranged between 2 to 20 birds. Second, the semi-intensive with the bird numbers typically range from the least ownership of 25 birds to hundreds. Finally, there is professionally managed intensive system. The number of chickens reared is varies from hundreds to thousands. The annual eggs productivity of intensive system reared-poultry is very high compared to that of extensive system (146 - 260 eggs vs. 37 - 47 eggs) and mortality is typically lower than the other two rearing systems as well.

Keywords: benefits, native chickens, farming systems, Indonesia

S14-0009 Characteristics and significant contribution of small-scale family poultry production in developing countries

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The term “family poultry” used to describe the full variety of small-scale poultry production systems that are found in rural, urban and peri-urban areas of developing countries. Being called ‘family poultry’, ‘smallholder poultry’, ‘scavenging poultry’, or “village poultry” the different systems of poultry rearing with various levels of intensification are now adopted by poor, marginal as well as richer members of the society with intensification according to their economical status and requirements. The term “poultry farming” refers to the raising of domesticated birds such as chickens, turkeys, ducks, and geese for the purpose of farming meat or eggs for food. Worldwide, poultry consists of chickens (90.55%), ducks (5.53%), geese and guinea fowl (1.67%), turkeys (2.09%), and other poultry (0.15%). There are four broad well recognized family poultry farming systems in developing countries. They are (1) Free-range extensive system, (2) Backyard extensive system, (3) Semi-scavenging system, and (4) Small-scale intensive system. Characteristics of them met the following criteria: (1) production/farming system, (2) other livestock raised, (3) flock size (adult birds), (4) poultry breeds, (5) source of new chicks, (6) feed resource, (7) poultry housing, (8) access to veterinary services and veterinary pharmaceuticals, (9) mortality, (10) access to reliable electricity supply, (11) existence of conventional cold chain, (12) access to urban markets, (13) products, and (14) time devoted each day to poultry management. Family poultry makes a significant contribution to poverty alleviation, food security, empowerment of women and wildlife conservation in developing countries. Small-scale family poultry production has been of great help in easing the food situation as well as provides a subsidiary income amongst many poor in many developing countries.

Keywords: characteristics, contribution, farming poultry farming systems, developing countries

S14-0010 Active surveillance for avian influenza in selected areas of Bangladesh

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Bangladesh experienced first outbreak of highly pathogenic avian influenza (HPAI) in early 2007. A surveillance program for avian influenza in three selected areas was conducted jointly by FAO and PROSHIKA. The surveillance included clinical surveillance, flash reporting, blood collection for serological, swab collection for virological surveillance and collection of fecal samples from migratory birds. Ten epidemiological units from each of the selected areas consisting of 7459 households were selected. Staff recruitment and training, development of surveillance form, data collection were the main activities. Blood sera collected from epidemiological units were clotted, separated sera, collected in Eppendorf tubes until shipment to the Laboratory for detection of antibody against H5N1 virus. Cloacal /tracheal swabs were sent to the laboratory from the dead/ sick birds. During the surveillance works, avian influenza outbreak occurred in backyard chicken also. Vaccination and bio security were poor. Routine vaccination against infectious diseases, improvement of bio security and veterinary services, strict follow-up are the important areas for controlling HPAI and other diseases.

Keywords: biosecurity, swab, backyard, vaccination

S14- 0011 Optimization of dietary protein concentration through balanced amino acid nutrition for Vanaraja (a backyard variety) chicks during nursery rearing

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The response of Vanaraja (a backyard variety) chicks during nursery rearing (0 to 6 weeks of age) to reduced dietary protein concentration was evaluated in 2 experiments. In experiment 1, graded levels of dietary protein were evaluated. A total of 275 day-old chicks were divided into 5 groups with 11 replicates of 5 chicks each and fed 19, 17 and 15% CP, the latter two CP levels with and without protease supplementation (15000 u/kg). The contents of lysine (5.2% of CP) and total sulfur amino acids (4.1% of CP) were maintained in a fixed proportion to protein content. In experiment 2, reduced dietary protein levels with and without balancing for amino acids (AA) were evaluated. A total of 630 day-old Vanaraja chicks were divided into 7 groups with 15 replicates of 6 chicks each and fed 18, 16, 14 and 12% CP, the latter 3 CP levels with and without balancing for critical AA (lysine, methionine, threonine and tryptophan). In experiment 1, body weight (BW) and feed conversion efficiency (FCE) were similar in 19 and 17% CP groups, while 15% CP reduced ($P<0.05$) BW and FCE. Feed intake (FI) was higher in 17 and 15% CP groups. Protease supplementation showed no effect. In experiment 2, BW was similar between 18 and 16% CP, but was significantly depressed at 14% CP, while AA balancing improved BW on par with that of 18% CP upto 3 weeks of age. But later, BW was improved moderately with AA balancing. BW was drastically depressed at 12% CP, which improved marginally with AA balancing. FCE was reduced and giblets and abdominal fat weights increased at lower CP levels, which were improved by AA balancing at each level of CP. Serum protein content decreased at 14 and 12% CP, whereas no effect was observed on serum cholesterol content, ND titres and dry matter digestibility. The results indicated the possibility of reducing dietary protein level (upto 4% till 3 weeks and 2% during 0-6 weeks of age) by balancing AA in the diet of Vanaraja chicks.

Keywords: protein, amino acid, Vanaraja diet, nursery rearing

S14- 0012 Analysis on the decision making mode of Chinese farmers' breeding scale structure perspective

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In recent years, our country is in the transforming period which changes the Traditional Cultivation into Modernizing Breeding. Large- scale breeding is become the important feature of breeding industry in future. Based on farmers' behavior theory, the thesis analyze the topic of farmers' breeding scale with Quantile regression model. The random sampling data of large scale breeding laying hens in 2015 Contains 678 valid samples, which is extracted from the Industrial and Technological System of National Laying Hens. The result has been listed as follows: (1)Individual Characteristics: As the breeding scale expands, negative effects of the farmers' age become stronger; the education level mainly has obvious positive effect the breeding scale expansion on small and medium-sized farmers, experience has significant effect on large scale breeding. (2)Family Characteristics: As the breeding scale expands, labor, household income and the income of laying hens breeding have much more stronger influence on large scale breeding positively. (3)Breeding Characteristics: In medium and large- scale breeding farms, renting land makes more important impacts. The standardized demonstration of breeding farms mainly has positive effect on small and medium farmers. (4)Outside Environment: As the enlargement breeding scale, strict environment policy and average breeding scale in a village have strong influence on large scale breeding positively. The policy implications as follows: (1)Improve the farmers' educational level through training to improve the development of large- scale breeding. (2)Focus on key cultivating object who is younger with sufficient household labor force for large- scale breeding. (3)Promoting land circulation is good for further development of large scale breeding farmers. (4)We should focus on the standardized demonstration roles of small and medium breeding scale farmers.

Keywords: farmers, large scale breeding, scale structure

S14-0013 Bio-security, diseases, predation and myths are the major constraints in rural poultry sector growth in developing countries. A- case study

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Rural poultry play key role in nutritional security of the poor; hence intense efforts are going on to develop this sector around the globe. Pakistan, like many other developing countries, has huge population living in rural areas that raise chicken to fulfill their nutritional needs however this sector is not that contributing to the food supply as it has the potential to do so. Thus this study was planned to dig out the possible causes behind slow growth in this sector. So we conducted a survey involving 302 small- scale- poultry- growers respondents (30- 40 chicken per flock) from 35 villages of province Punjab. We found high mortality losses among the flocks, out of which 39% were due to disease outbreaks. The major diseases were; Newcastle disease (93%), Enteritis, Fowl Pox, Parasitic infestation and Typhoid fever. Most disease- diagnosis were done by the farmers themselves (37%) while getting services of the Veterinary doctors were minimal (7%) hence most chicken (90%) died during the outbreaks. No bio-security measures other than vaccination against contagious diseases were adopted while 27% of the flocks were never vaccinated. Predation was also one of the major causes of losses and among the predators dog attacks were the most common (41%) followed by attacks from snakes (21%), foxes, cats and mongoose. To avoid predation though 30% of the farmers rely on killing of the predators, 14% protected their flock by improving housing and majority did not have means to handle this problem. Most farmers (93%) earned high profits on sale of poultry products during winter due to perception of the consumers that eating poultry products is more suitable during winter and vice versa for the summer. We concluded that in order to improve small-scale-poultry production there is need to focus upon improving housing, disease diagnosis, treatment and prevention, and also educating communities about their seasonal dietary needs and usefulness of poultry products in this respect.

Keywords: rural poultry, food security, major diseases, marketing barriers

S14-0014 Comparative evaluation of Kamrupa, Vanaraja, Pb2x Indigenous and Indigenous chicken in rural condition of Assam and North East India

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Purpose and objective of the experiment: The people of Assam usually rear some indigenous bird in backyard system. Kamrupa is a dual type new variety and Pb2 is a broiler breed. In this study the comparative performance of Kamrupa, Vanaraja, Pb2x Indigenous and Indigenous chicken is evaluated for rural adoption. Experimental Design: In the present study 2400 nos of chick were brooded in deep litter for 21 days after which the chicks were distributed to 40 farmers to be reared in scavenging condition with an allocation of 15 chicks from each group. The birds eat different items during scavenging apart from some broken rice and rice polish provided by the farmers. The traits recorded were body weight, conformation traits, average age at maturity, egg production, egg weight, egg quality, carcass traits, fertility, hatchability and economics. For egg quality, carcass traits, fertility and hatchability study 400 eggs, 80 adult chickens and 2000 eggs were taken. For economics study the cost of different items were considered as per prevailing market price. The data were analyzed following the standard statistical methods. Results: The body weight (g) from day old to 40 weeks ranges from 28.92 ± 1.80 to 2310.50 ± 175.70 g. The conformation traits ranges from 3.95 ± 0.36 cm to 69.72 ± 3.10 degree. The average age at maturity, egg production upto 32 and 40 weeks, annual egg production and egg weight at 32 weeks and 40 weeks of age ranged from 23.50 ± 1.65 nos to 218.26 ± 4.15 days. The egg quality traits ranged from 0.081 ± 0.001 to 81.35 ± 2.62 . The values for different carcass traits ranges from $64.96 \pm 3.40\%$ to 2240.23 ± 215.90 g. The fertility, hatchability and mortality is generally better in Kamrupa than the other groups. Net profit per bird was calculated to be slightly higher in Kamrupa bird (Rs 153.70) than that of Indigenous bird (Rs 128.20). Conclusion: Performance of Kamrupa bird is found better than that of other groups for most the traits except body weight. **Keywords:** Kamrupa bird, vanaraja bird, pb2x indigenous bird, indigenous bird, extensive system

S14- 0015 Research for new policy options: raising village chickens to tackle food and nutrition insecurity

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Taking a One Health approach, our research “Strengthening food and nutrition security through family poultry and crop integration in Tanzania and Zambia” explores options to increase the efficiency of family poultry and crop value chains. In a context of climate change, increasing poverty and food insecurity, the importance of improving food production, nutrition and health among vulnerable populations is a matter of urgency, especially for women and children. In sub-Saharan Africa, as in most parts of the world, women in rural areas make a major contribution to village poultry and traditional crop production and assume much of the responsibility for the food security, nutrition and health of their households. The randomised controlled trial involves five wards (three in Tanzania and two in Zambia), each with approximately 300 households with children under the age of two years at the time of enrolment. The primary beneficiaries of this project are the rural poor across approximately 10,000 households, especially women and children. The project is making significant contributions to the social and biological understanding of options for improving childhood nutrition by improving and integrating family poultry and nutritious secondary crop production. It aims to strengthen food and nutrition security in project communities by increasing the availability, accessibility and utilisation of foods of both plant and animal origin at a household level. It demonstrates the benefit of a multidisciplinary approach by engaging all key sectors from national to community levels in project implementation and seamlessly integrating social science research into all activities. This research explores opportunities to raise stakeholders’ awareness of women’s contributions to household and community food security while also contributing to the social sciences and facilitating policy options across both health and agriculture.

Keywords: village poultry, one health, nutrition, value chain

S14– 0016 Comparative growth performance of four varieties of naked neck chicken maintained in Pakistan

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Present study investigated growth performance of four naked neck varieties. A total of 320 day old naked neck chicks were arranged according to completely randomized design and divided into 4 treatment groups having 5 replicates of 16 birds each. Treatment consisted four different varieties, based upon their plumage pattern viz. black, white with black tips, light brown and dark brown. The experimental birds were kept in cages in a well-ventilated open sided poultry house up to 8 weeks of age and fed layer diet manufactured according to NRC standards. Results revealed that Light brown and dark brown variety of naked neck showed significantly highest ($P<0.05$) body weight and total weight gain than that of other varieties whereas times of gain and average FCR were observed to be significantly higher in dark brown naked neck. However, feed intake and overall livability % remained non-significant among different varieties of naked-neck. Based on the findings of this study it may be concluded that dark brown variety of naked neck exhibited better growth rate and FCR than three other varieties.

Keywords: naked neck, plumage, growth, Pakistan

S14– 0017 Productive performance and egg quality traits of Tunisian local laying hens

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Few studies are made regarding Tunisian local laying hens' performance and egg quality. However, this farming poultry may contribute to sustainable agriculture and rural development. Thus, the current study aims to assess egg production and egg quality characteristics in local hens. A total number of 96 Tunisian local laying hens of 8 phenotypes (red, brown, barred grey, white, black, beige, necked neck and crested hens, of 18 weeks of age, is used in this study. Each phenotype was housed in 1 or 2 outdoors' chicken runs. During a period of 18 weeks, laying rate (LR), feed intake (FI), feed efficiency (FE), egg weight (EW), egg length (EL), egg width (EWD), albumin weight (AW), yolk weight (YW), yolk length (YL), shell weight (SW), and shell thickness (ST), were evaluated and were significantly different ($P<0.001$). Initial and final live body weights were controlled. Mean birds weighed about $14967\text{g} \pm 247,06\text{g}$. Laying rate averaged 44.92% with a minimum of 2.52% at an age of 18 weeks, and a peak of 72.46% was detected around an age of 28 weeks. Mean values of (FI), (EW), (EL), (EWD), (AW), (YW), (ST), (YL) and (SW) were about 126.4g/day/bird, 49.35 g, 52.57 mm, 40.27 mm, 26.91g, 14.91g, 0.65mm, 38.84 mm, 6.057g, respectively. Roche scale yolk color of most phenotypes' groups was about 5. To conclude, Tunisian local laying hens is characterized by a clear phenotypic heterogeneity which influencing egg production and egg quality. Furthermore, this study shows that local laying performance are relatively low.

Keywords: Tunisian local laying hens, performance, egg quality

S14-0018 Potential of village chicken production to improve livelihood of rural women

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A cross-sectional study was conducted in Lilongwe District, Malawi, with the objective to evaluate socio-economic characteristics of rural farmers engaged in village chicken production. A pretested structured questionnaire was administered to a random sample of one hundred and forty-eight (148) farmers. A three stage cluster sampling technique was used to select two sections, and two group headmen of Mitundu and Mkwinda Extension Planning Areas, from where households were sampled using random number generator. These represented 40% of the highest sample size determined by using standard deviations of five parameters from one of the studies that was carried out in the area. The data collected was subjected to descriptive statistics for qualitative and quantitative variables using SPSS 23. The results showed that the majority (98.4%) of chicken keepers are female-headed households. Few households (1/4) do not own land, and for those that have land, 25% of female-headed households own half a hectare or less. By contrast, only one-sixth of male-headed households own half a hectare or less and 11% possess above 2 hectares whereas with female-headed households, it is only 7% that possess land above 2 hectares. Fifty-five percent of female-headed households fall within the lowest category (MK50000 or less) of annual on-farm income; none of the female-headed households earn annual income above MK250000 (US \$1=MK747.51), while 22% of male-headed households earn above MK250000, out of which 12% earn above MK650000. Despite this demography, almost all female-headed households keep village chickens, thereby providing an opportunity for improving village chicken production that could enhance the economic potential of female-headed households. That is, improving productivity of village chickens can help empower rural communities, especially women who are custodians of the chickens.

Keywords: village chicken production, rural women empowerment, livelihood, livestock ladder

S14-0019 Performance of local pullet chickens fed diets of graded levels of raw or boiled pigeon pea (Cajanus cajan (L) Millsp.) seed meal

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A total of 324 day-old local chicks were used to determine the performance of local pullet chickens fed diets of graded levels of raw or boiled pigeon pea (Cajanus cajan (L) Millsp.) seed meal. The experimental design was 2 x 5 factorial in completely randomized design (CRD). Raw or boiled (in water for 30 minutes) pigeon pea seed meal (PSM) was milled to pass through a 2 mm sieve and included at 0%, 15%, 20%, 25% and 30% in the chicks (1-56 days) diets. Each treatment diet had three replicates and 12 pullet chicks per replicate. The experimental diets were isoenergetic and isonitrogenous with PSM replacing part of maize and soybean meal in the diets. Parameters measured were weight gain, live weight, feed and protein intakes, feed conversion ratio and protein efficiency ratio. Data collected were subjected to analysis of variance (ANOVA) at 5% level of probability while significant differences among treatment means were separated using Duncan's New Multiple Range Test. Results showed that raw PSM in the diets significantly ($P < 0.05$) increased daily feed intake (8.95 g/b) over boiled PSM (7.87 g/b) while other performance indices were not significantly affected. Inclusion of 25% PSM in the diet significantly ($P < 0.05$) depressed feed intake (8.08 g/b) of the local pullets more than control (8.95 g/b) and 20% PSM (8.45 g/b). Daily protein intake was significantly ($P < 0.05$) reduced by all levels of PSM in the diets compared to the control diet. Raw PSM inclusion as 15% of the diet gave significantly ($P < 0.05$) higher pullet live weight (132.33 g) at 56 days achieved with significantly lower feed (8.79 g) and protein intakes than 20% raw PSM. Boiled PSM at all the inclusion levels significantly depressed daily feed intake of local pullets compared to raw PSM. The conclusion was that raw or boiled PSM could be included up to 30% in the diet of local pullet chickens without adverse effect on performance.

Keywords: diets, Local Pullet chickens, performance, pigeon pea seed meal

S14- 0020 Using an innovation platform for the introduction of improved tropically adapted germplasms to small scale family poultry producers in Nigeria

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An Innovation Platform (IP) approach was adopted for the introduction of improved tropically adapted germplasms (iTAG) to small scale family poultry (SSFP) producers in 5 agroecological zones in Nigeria. National (NIP) and sub-national (SNIP) meetings discussed challenges and opportunities in adopting iTAG. The NIP was facilitated by a change organization from Kenya while the SNIPs were facilitated by project staff who had been trained by the change organization. A baseline survey was conducted with 1200 respondents in 60 villages. All data were collected electronically with the Open Data Kit (ODK) installed on Lenovo TAB 2 A7-30 tablets. Data were submitted into a repository developed by the International Livestock Research Institute (ILRI). Field officers were given a 10-day training on the use of the tablets for data collection, and for uploading data and geographical position system coordinates in real time to the central server at ILRI. Mean flock size was 21. Flock size for 95 percent of households (HH) \leq 50. 80 percent of HH reared only local chickens. The 32 participants at NIP comprised 41% value chain (VC) actors, 41% VC facilitators, 18% support institutions. A total of 101 people participated in the SNIPs comprising 31% VC actors, 53% VC facilitators 13% support institution and 3% community leaders and NGO. 12 action areas were identified as required for the successful adoption of iTAG: input supply, housing, community linkage, cooperatives organisation, policy support, farmer training, marketing, brooding to 6 weeks before distribution, obligatory veterinary services during brooding, extension services, finance and insurance, monitoring and evaluation. It was concluded that the IPs made the stakeholders aware of the challenges and opportunities inherent in the iTAG innovation for the SSFP value chain.

Keywords: family poultry, adapted germplasms

S14- 0021 Effects of hormone and mating on laying performance of female pigeon

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In the present study, we investigated the hormone changing pattern of female pigeon in laying cycle, and studied effects of mating and exogenous hormone on pigeon laying. The ranges of variation of FSH, LH, PRL, E2, and P in normal laying pigeon were respectively, 0.02 to 0.18 mIU/mL, 0.03 to 0.42 mIU/mL, 0.02 to 0.295 ng/ml, 30 to 187 pg/ml, and 0.03 to 0.22 ng/ml. While in non-laying pigeon, except E2, the other four hormone concentration was just only one lower level. Differed from normal laying pigeon, preovulatory follicles and even small yellow follicles were not seen from the slides of non-laying pigeon. In non-laying pigeon, the whole volume of oviduct was much smaller, and villus atrophied, and distance between villus was enlarged. Mating can induce hormone changing in normal laying pigeon. Mating, or in association with other pigeons are very important to mature of pigeon's follicle. Injection of FSH and LH has no effect on laying of single isolated pigeon. Therefore, successful mating and normal hormone changing pattern are both essential to regular pigeon laying. Try to group pigeons according to the gender early in young pigeon stage may promote the successful mating.

Keywords: pigeon, hormone, mating

S14-0022 Broiler feed brand preferences by small scale poultry farmers versus performance of starter broilers fed the farmers' preferred feed brands in Owerri, Zone, Imo State, South East, Nigeria

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The production and sale of 28 day old broilers, a business known locally as “brood and sell” is rapidly growing among small scale poultry farmers in Owerri zone, Imo state, South East Nigeria. Feed however, is a major budget item that affects profit in the business. It therefore, became imperative to evaluate the preferences of farmers for the different starter broiler feed brands versus the performance of such broilers on these feed brands. Purposively structured questionnaires were distributed to forty eight small scale poultry farmers in seven local government areas in the zone. Based on the responses from the farmers, a 28 day feeding trial was carried out at our station using 210 day old Marshal broilers to determine their performance on feed brands preferred by the farmers. Results of the survey indicated that five feed brands coded TF, VF, AF, HF and ZF were actively distributed in the zone. VF brand was the most preferred (49.10%) and TF second most preferred (29.07%) while HF brand was the least preferred (3.66%). Results of the feeding trial indicated that TF recorded the best body weight gain (495.22g), feed conversion ratio (FCR), 2.53 and 0.00% mortality while HF recorded the least body weight gain (214.29g), poorest FCR (5.57) and highest mortality rate (14.28%). The VF brand mostly preferred by the farmers from our survey recorded 469.04g in body weight gain, 2.87 FCR and 0.00% mortality. TF which outperformed other brands however, cost much more per kg which from our survey explains it being second in preference to VF. Preference of feed brand by small scale poultry farmers in Owerri zone, from the results obtained is influenced by cost but the quality of such feed brand was also a major consideration.

Keywords: small scale poultry farmers, broiler feed brand, starter broilers, Owerri

S14- 0023 Development and evaluation of productivity of ‘CARI shyama’ – the replica of Kadakanath breed of Indian native chicken

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Lack of high productivity scavenging chicken germplasm having adaptability to local environment, considerable resistance to tropical diseases, self propagating ability, look of native birds for acceptability and satisfy consumer preference for eggs and meat of indigenous/local bird and cultural linkage is one of the most important constraint of traditional free range poultry production system. Improved native breeds or a cross of native breed with exotic have been advocated as bird of choice for replacement. CARI Shyama has been developed by crossing the Indian native breed Kadakanath with high yielding CARI Red which possesses essential characteristics of scavenging chicken. The genes responsible for tropical adaptability viz. Slow feathering (K), Non-inhibitor dermal melanin (id) and Fibromelanosis (Fm) present in Kadakanath make the CARI Shyama capable to bear stress of hot and humid tropical environment. Further, Fibromelanosis causes increase in muscle protein, decrease in fat and thin collagen fibre which is the best meat characteristics. Birds attain the weight of one kilogram at nearly 15 weeks of age and males and females weigh nearly 1461 and 1104 g, respectively at 20 weeks of age. Under village conditions attain the age of sexual maturity at nearly 167 days and produce annually (72 weeks of age) 210 deep brown thick shelled eggs of 53 g. CARI Shyama is the true replica of desi fowl, which is suitable to substitute low producing non- descript desi fowl. It can play vital role in combating the problem of protein deficiency in rural areas due to higher quantity of protein in its meat along with better egg production potential.

Keywords: Kadakanath, fibromelanosis, scavenging chicken

S14- 0024 Small scale poultry rearing in Tanzania – Subsistence to surplus production for increase income and improve food and nutrition security

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In Tanzania there are about 36.2 million chickens, of which almost 95% are local chickens are reared by rural households. Small scale poultry rearing is crucial and play significant roles in poverty alleviation, improve food and nutrition security, increase income and improve the livelihoods of rural and disadvantaged farmers especially women in Tanzania. BRAC Maendeleo Tanzania (BRACMT) recognises it and has been implementing a poultry development model to provide the entire package needed by small farmers since 2012. BRAC selected 3000 poor small and marginal farmers with a special focus on women with disability and living with HIV/AIDS who require support as its project participants. The project provides comprehensive support including high quality inputs, capacity building and technology transfer, extension services and linking with market. A study was designed and conducted for a randomized control trial involving 126 farmers (treatment and control) in Tanzania. We created a longitudinal panel dataset from farmers that were tracked throughout the study for three years of project period to compare any improvements in a number of selected indicators (productivity, income, food security and nutrition). Results indicated that there were improvements in productivity (550%), income (65%), and food and nutrition (egg & meat 41%, HFIAS 4.81) security among farmers in the treatment farmers. Factors that were significant determinants of productivity and income were training, extension services, inputs, vaccination services, extension services and market link.

Keywords: small scale poultry, subsistence to surplus, quality inputs, capacity building, productivity, income, nutrition

S14- 0025 A preliminary assessment of the perceptions and sensory acceptability of village chicken meat among residence of Khaya Ward, Kwazulu-natal, South Africa

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The study assessed consumer perceptions towards village chicken meat consumption and its sensory acceptability in Khaya ward, KwaZulu-Natal Province, South Africa. We conducted the study in three parts using a survey, focus group discussions and sensory evaluation. A questionnaire written in isiZulu which is the local language spoken in KwaZulu-Natal was administered to 60 household representatives. Similar number of participants also took part in the sensory evaluation while three focus group discussions were conducted to determine consumer's perception on village chicken meat consumption. Village chicken purchase and production were the main source of chicken procurement in the study area. An estimated 95% of the respondents agreed to consuming village chicken meat and 62% considered the inclusion of village chicken meat in household diet as essential due to the perceived nutritional and organoleptic benefits. Our findings revealed that village chicken meat was more preferred meat-type than the broiler meat. Based on the student t-test, there was a significant difference ($P \leq 0.05$) in the colour of village chicken meat when compared to the control (broiler meat). A relatively lower proportion of respondents disliked the taste, texture and aroma sensory attributes of village chicken meat and broiler meat. This contributed positively to the overall acceptability of both meat types, which was similar and very high among the participants. Nevertheless, there is a need for education programmes in the study area aimed at training households on general flock management for improved village chicken production and nutritional benefits. This will boost the use of village chickens as a source of high quality and easily digestible protein.

Keywords: broiler, rural households, sensory evaluation, village chicken

S14- 0026 The experience of small-scale farmers in poultry production: analysis of risks and benefits

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The poultry industry is widely recognized as setting the pace for livestock production. The particular aspects of poultry production, from its technologies to its contract model and farmer payment mechanisms, are now being adopted globally on the heels of an increasing trend in meat consumption. This market expansion and growth presents many opportunities, including potentially for small-scale farmers worldwide. The question then is do small-scale family farms benefit from inclusion in a vertically integrated poultry value chain? To understand their independent contractor experience of raising poultry, this paper examines the economic performance and risk exposure of several farmers in the southeastern United States. Quantitative and qualitative data collection methods are used to compare farmer experience of risks and benefits in broiler production contracts to what is already understood in the existing academic literature. This paper expands the academic and industry dialogue of the role of smallholder farmers in poultry production by assessing at a greater level of detail risk exposure and benefit gain from contract relationships with integrators. An in-depth case-study methodology is utilized, which includes highly detailed results from 20 farmers in 7 different states in 2015 - 2016. The data collected is presented along with analysis of 21 found risk or benefit elements including individual farmers' experience of consistency in pay, costs of production, revenues for over time, autonomy and farm management, and several others. Thus the hypothesis is tested that: A broiler production contract is a stable, implicitly long-term and low-risk investment for a small-scale farmer who is a good manager. The results of this paper provide a rich platform for further discussion about the potential role of small-scale family farmers in globally expanding poultry production.

Keywords: small-scale farmer, risk, benefit, costs of production, revenues, case-study, poultry production

S14- 0027 A preliminary assessment of the perceptions and sensory acceptability of village chicken meat amongst households of Khanya Village, KwaZulu-Natal, South Africa

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The study assessed consumer perceptions towards village chicken meat consumption and its sensory acceptability in Khanya village, KwaZulu-Natal Province, South Africa. The study was conducted in three parts using a survey, focus group discussions and sensory evaluation. A questionnaire written in isiZulu which is the local language spoken in KwaZulu-Natal was administered to 60 household representatives. Similar number of participants took part in the sensory evaluation, while three focus group discussions were conducted to determine consumer's perceptions on village chicken meat consumption. An estimated 95% of the respondents agreed to consuming village chicken meat and 62% considered the inclusion of village chicken meat in household diets as essential due to the perceived nutritional and organoleptic benefits. Our findings revealed that village chicken meat was the more preferred meat-type than the broiler meat. Based on the student t-test, there was a significant difference ($P \leq 0.05$) in the colour of village chicken meat when compared to the control (broiler chicken meat). Taste preferences for village chicken meat created an increased demand for village chickens enabling a premium price to be obtained from the sale of village chickens. Nevertheless, there is a need for education programmes in the study area aimed at training households on general flock management for improved village chicken production and the nutritional benefits of village chicken meat.

Keywords: rural households, sensory evaluation, village chicken, broiler chicken

S14-0028 Effect of inclusion of brewer's dried grains on growth performance of native chicken reared in mud floor system

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A study was conducted to evaluate the effect of inclusion of Brewer's Dried Grains (BDG) on growth performance of Native chicken in mud floor system of rearing at University Research Farm, TANUVAS, Tamil Nadu, India. A total of 320 native chickens of eight weeks old were housed in mud floor and were randomly divided into four treatment groups with 4 replicates of 20 birds each. An Iso-nitrogenous and iso-caloric experimental feeds were prepared by including BDG at zero per cent (control, T1), 3 per cent (T2), 6 per cent (T3) and 9 per cent (T4) level in the feed and fed ad libitum for a period of four weeks under standard managemental conditions. The parameters like body weight, weight gain, feed conversion ratio (FCR), livability were recorded. Highly significant results were observed ($P < 0.01$) in body weight and weight gain. Higher body weight (g) was noticed in 6 per cent inclusion of BDG (1111.01 ± 9.47), followed by comparable body weights in 3 per cent and 9 per cent level than control (T1). Similarly the body weight gain also better in 6 per cent inclusion of BDG (325.57 ± 1.20). The FCR and livability did not show any significant difference. It is concluded that the inclusion of BDG will enhance growth performance of Native chicken reared in mud floor with better return and reduce cost of production.

Keywords: native chicken, body weight, brewery industry waste, mud floor

S14- 0029 Consumers erroneously perception on nutrition of free range eggs

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Alternative production systems, such as cage-free or free-range egg production, have been growing in numbers to satisfy recent changes in consumer demands. Today's consumers have an increased desire for eggs produced in more extensive poultry production systems, such as free range egg production, because of concerns about their health and the use of the cage environment. Currently, consumers have little knowledge and a lot of beliefs about eggs and egg quality. Their ideas on the ideal egg differ so much that it is not possible to define one or two ideal eggs. Each consumer seems to have his/her special preference for a certain combination of either external or internal quality characteristics of eggs such as freshness, type (free range or not), yolk colour, buying place, size or weight and shell colour. Consumers also erroneously believe that the following four common factors affect the nutritive value of eggs. They are: (1) fertile eggs are more nutritious than infertile eggs; (2) brown shell eggs are more nutritious than white shell eggs, or vice versa; (3) an egg with a deep yellow yolk colour is higher in nutritive value than those of a lighter shade and (4) free-range eggs are higher in nutritive value than eggs from cages. Although a tasting panel indicated a preference for free-range eggs when they were fresh and they could see what they were eating, blindfolded they could not tell the difference between those produced on range or in cages. No influence of housing environment (range or cage) on egg levels of Vitamin A or Vitamin E. However, β -carotene levels were higher in the range eggs which may have contributed to the darker colored yolks compared to the cage eggs. Eggs from a range production did have higher levels of total fat than eggs produced by caged hens, but they did not have higher levels of cholesterol.

Keywords: consumers, perception, free range eggs

S14-0030 Mother Brooder Unit concept for better market penetration of SASSO colored birds in Rural India

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Consumer preference in the “Wet market” is for colored birds in the sub continent of Asia in general and India in particular. The 3 weeks & above grower chicks are field ready and are preferred for backyard rearing. SASSO from France is one such commercial multicolored bird available in India that addresses the requirement of rural farmers of India. Suitability of SASSO colored broilers for rearing at Mother Brooding Unit (MBU) was studied at a commercial farm in Coimbatore, India in 2014. A batch of 3000 SASSO day old chicks from Ayilas Farms Hatchery, Coimbatore was reared for the purpose of sale by 3 weeks. Electric brooders, locally available commercial feed and vaccines for ND and IBD were administered. Chicks were reared in open sided deep litter shed on 0.5 sft floor space per bird. Part of the batch (235) birds were individually studied on 21 day for commercially important traits such as live weight, feather color pattern, shank length, shank color and tail length and their inter relationships. Male: Female ratio was 50:50 with an average live weight of 284 g. Multicolor feather pattern was noticed with 46% brown, 26% brown mixed and 18% black. Overall, 58% birds had yellow, 33% grey and 9% had black shanks. Only shank length was positively correlated with live weight. Customer response to SASSO colored chicks was good due to attractive phenotypic features. Batch economics revealed that total cost of production was INR 79500 for a batch of 3000 day old chicks. Out of this, chick cost was INR 36000, cost of 1500 kg feed constituted INR 37500 and INR 6000 as other expenses. Gross returns from 21 days old grower birds was INR 99840 resulting in 1.25:1 benefit to cost ratio. Thus, the profit per chick placed was INR 6.78. In a year with 10 such cycles the profit realization per month would be INR 16950. Such units in in Rural India could address food, nutrition and financial security of small, marginal and landless farmers and farm women.

Keywords: Mother Brooder Units (MBU), SASSO colored birds, wet market, BC ratio, sustainable

S14-0031 Supply chain management of country fowls– a farmer initiative in India

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Backyard poultry accounts for 15-20 per cent of meat and egg supply chain in India and has a niche market even among the city dwellers. The negative features like broodiness, low egg production and fibrous meat quality has not deterred the enthusiasm of the growers, while the unique flavour and health benefits have attracted consumers despite double the price of white broilers. Keeping these facts in mind, a school dropout youth farmer has started a home grown country eggs aggregation model to meet demand for country chicks among the rural farm households in Mandya district of Karnataka. The youth has set up a full-fledged hatchery with a back-up generator having three setters with 40000 egg holding capacity and two hatchers with 12000 capacity. The network comprises of about 300 farmers spread over about 20 villages in a radius of about 50 kilometres. Each village has an unemployed youth identified and entrusted with the responsibility of collecting breeding eggs once or twice a week who in return receive an incentive of INR 2 per egg. These eggs are offered 20-25 per cent premium price on the spot and transported in closed bamboo baskets layered with paddy husk. Each egg is marked with farmer and village code for tracking the fertility, hatchability and farmers loyalty. The village-wise collected eggs are aggregated by transporting in a van on a designated route. The day old chicks are graded, vaccinated and delivered at farm gate against advance bookings at INR 32-35 per chick. The hatchery is managed by four trained youth and the network provides technical advice to beginners. The forward linkage of supply chain is managed by the group serving as lifters to wet markets in cities and towns. These local poultry birds of 3 months age are sold at Rs. 150 per kg in rural areas and the same would fetch Rs. 250 in retail chicken shops. This offers a ‘win win’ situation to all stake holders along the supply chain of country fowls.

Keywords: supply chain, aggregation, country fowls, wet market, network

S15- 0001 Indian poultry industry: current status, practical challenges and opportunities

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The poultry industry in India is one of the important contributors to the economy of rural and semi-urban India. India is ranked the 3rd largest egg producer and the 4th largest chicken meat producer in the world. The organized sector of poultry industry is contributing 70 per cent of the total output. The broiler and layer industries are well dominated by southern states in India. Indian National Sample Survey Organization, in its 68th round report showed that in urban areas the demand for egg and chicken meat increased from 34 and 9 per cent to 37.6 and 27 per cent respectively during the last two decades. FAO also predicted that 42 per cent of meat that will be consumed worldwide by 2020 will be chicken meat, overtaking pork and beef. Increasing population, growing demand for convenient foods, awareness about inclusion of animal protein and rising per capita income are the major factors propelling the growth surge of Indian poultry industry. The contribution of livestock sector to India's GDP is \$ 47.33 billion during 2010-11 with the value of output from poultry sector being \$ 8.26 billion. During 2014-15, poultry population in India was estimated at 729.21 million and egg and chicken meat production has reached 697.31 million and 2.68 million tonnes respectively. According to Indian Council of Agricultural Research vision 2025, an increase in per capita availability of one egg will generate 50,000 more jobs. The major key issue of concern in the chicken meat sector of India is the processing status to ensure optimum quality and hygiene levels. Emerging and remerging diseases, potential environmental hazards such as poultry waste management, dead birds disposal, inadequate linkage between research and development organizations and industry are the biggest challenges to the growing Indian Poultry Industry.

Keywords: Indian poultry industry, present status, challenges and opportunities

S15-0003 A value chain analysis as a tool to evaluate highly pathogenic avian influenza (HPAI) intervention strategies

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A value chain analysis of poultry chain through the market channels is developed and combined with the information of highly pathogenic avian influenza (HPAI) transmission. It can be used to predict the effects of intervention strategies for HPAI control. The first step in development is a description of the main characteristics of the Western Java poultry value chain. It includes a description of relevant stakeholders, the number and value of poultry transferred between stakeholders and the governance of the value chain. By this innovative methodology, this study explained the relationship between the structure of the value chain and mechanisms of disease transmission. The developed value chain analysis provides a rational and systematic framework for describing the supply chain and epidemiological situation before intervention. It enables the simulation, both in overall economic terms as well as in HPAI transmission terms of possible intervention methods. The conceptual model will be parameterized for two important value chain scenarios for the Western Java situation: the traditional and the modern channel for poultry supply. Each scenario is illustrated with case examples. This study shows that the developed value chain analysis is a robust model that can be used to evaluate intervention strategies.

Keywords: value chain analysis, HPAI, supply chain, market channel, intervention strategies

S15-0004 Evaluation of some factors affecting floor egg production in broiler breeder Ross 308 and its economic effects

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The effect of some variables including hen density, nest density, mating ratio and nest height on reducing of number of floor eggs was evaluated in a field test in south khorasan, Iran with broiler breeder (Ross 308). Five flocks of broiler breeder belonging to 3 farms were used in experiment during 2008 to 2014. Data was recorded an interval of weekly for 200 weeks. The software SAS 9.1.3 was used for statistical analysis. The effect of all studied factors on percentage of floor eggs was significant, statistically. The level of hen density higher than 6.5 as well as nest density higher than 4.5 had the highest effect on eggs laid on floor. The occurrence of floor eggs was large with the level of mating ratios grouped in 7.5 or higher. In assessment of effect of nest height, a constant height of 35cm increased the number of floor eggs during the rearing period, compared to the variable nest height of 15 and 35cm. This data estimated a loss of 7500 dollars in a flock with 69% production and 11.3% floor egg.

Keywords: broiler breeder, Ross 308, floor egg, hen density, mating ratios, hatchability

S15-0005 Securing the financial stability of poultry producing enterprises: Problems and mechanisms

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The development of poultry production in Russia takes place in ambiguous and dynamically changing economic environment. Constantly growing inflation level is forming financially debt type of poultry enterprises characterized primarily by insufficiency of internal circulating assets and available sources of these assets. In the study presented financial stability of poultry enterprises was assessed using universal integral criterion, value of net assets (VNA). Analysis of financial condition included over 600 enterprises during 2012-2014. The gradation of poultry enterprises based on the VNAs was revealed: 68% of enterprises with growing VNA and 32% with decreasing VNA. 20% of all enterprises studied had negative VNA. In 10% of enterprises liquidation committees were launched; 24 enterprises were fully discontinued. In addition, cumulative amount of net assets (73.2%) was concentrated in 8.5% of enterprises studied. The main factors deteriorating financial stability of poultry enterprises are: the lack of effective use of technological, labor and financial potentials; high inflation level; disparity between prices of productive resources and production for sale; dependence of poultry production on imported materials; low availability of credit due to the lack of necessary assets for pledge and high credit rates; insufficient state support due to incapacity of standard mechanisms to consider all specific peculiarities of different segments of poultry farming. Crisis of 2014, anti-Russian sanctions and reciprocal embargo showed that the main problem of present situation is imperfect mechanisms of state regulations of agrarian policy. The latter is recommended to be redirected towards sufficient and state-regulated financing during the period no less than 5 years, with state guarantee of fulfillment. State regulatory base should also become more valid and sustained.

Keywords: value of net assets, financial stability, internal circulating assets

S15-0006 Analysis of green intellectual capital and financial performance (study at poultry companies in Indonesia)

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Poultry companies have a very important role, especially in meeting the nutritional needs of the people of Indonesia. Products produced include chicks, nutrition, egg and chicken meat. To be able to survive in the global competition market, companies need to increase performance and maintain their business sustainability. This research is very important, because they are trying to assess the performance of the Green Intellectual Capital (GIC) and Finance Performance of poultry companies in Indonesia. GIC is an Intellectual Capital (IC) owned by companies that carry out Corporate Social Responsibility (CSR). IC measured by the Pulic method (1998), which consists of VACA, VAHU and STVA. CSR is measured with CSR ISO 26000. Financial performance measured by ROA. Research sample is purposive sampling with certain criteria. This is veritative descriptive research with quantitative approach verification. The data is secondary data obtained from the company's financial statements include sales, cost of production, direct labor costs, administrative salaries, and marketing costs. The analytic method used to test the hypothesis is multiple regression with 90% confident interval. Descriptive analysis of elements of GIC are compared to the standard value of Guilford (1956). The study found a positive effect of GIC on ROA. Based on the standard value of Guilford GIC value is high (above 80%). Partially STVA and CSR has no effect on ROA. VAHU and VACA has effect on ROA, but the contribution of VAHU is very low (near zero). The results of descriptive statistics showed the highest average values are: VAHU; 4.628000, ROA; 0.107431 VACA; 0.354062, STVA; 0.684625 and CSR; 0.31875

Keywords: green intellectual capital, VACA, VAHU, STVA, CSR, financial performance

S15-0007 Analysis of the consumption characteristics of processed quail egg products

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China's economy steps into the "new normal" phase, as the eggs industry is growing into a limited manner, which will rely more on innovation to drive its growth. Egg processing is an effective method to meet the diversified demands, while there is lack of consumption behavior analysis of processed egg products in the extant literature. Therefore, taking processed quail egg products as an example, this study is to analysis the consumer preferences, perceptions and purchasing behavior based on consumer purchase decision-making theory. The intention is to provide suggestions for producers, as well as to improve the effective supply of eggs market. The sampling frame was family consumers based in Beijing, which was organized by Center of Rural Household Economy in China. To generate a list of informants, a quota sampling method was adopted and 102 valid questionnaires had been received in 2015. The descriptive statistics show that: 1) The male consumers with higher income and younger age possess higher consumer preferences for processed egg products; 2) There is higher cognition of preserved quail egg than spiced and toasted quail egg; 3) The male consumers with higher income but older age spend more money on processed quail egg products; 4) The safety, taste, package sealing, nutrition, convenience of processed quail egg products attract more consumers' attention, while the packaging, edible methods, specifications and brand are opposite; 5) The main channel for consumption is the supermarket but the development space of network market is rather broad. In addition, the results of this study are that the producers of processed quail egg products need to: 1) raise consumer perceptions; 2) do market segmentation and positioning according to the consumer demands; 3) strengthen the brand development; 4) promote the construction of e-commerce channel.

Keywords: processed quail egg products, consumer characteristics, demand diversification, management decision

S15-0008 Why China's brand eggs market share is too low? An empirical interpretation based on the extension of the utility maximization model

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China's laying hen industry has developed rapidly in recent 20 years; while the market share of domestic brand eggs is still too low. Take the highest degree of brand city as an example, the share of Beijing's brand eggs was only 35% in 2015. The objective of this paper is to develop a conceptual framework that is able to better understand the underlying drivers of consumer choice of common eggs or brand eggs. Based on the consumer's maximum utility theory and commodity attribute of agricultural products, this paper intends to extend consumer's eggs utilities for four aspects: product, safety, hedonic and ecological value. This paper assumes that it is the difference of these four utilities that cause the different behavior for consumer to choose common eggs or brand eggs. This paper carried out an empirical analysis based on the Heckman Two-Step selection Model and used the data which came from a random sample survey in 2015 and the total sample size was 376 urban households in Beijing. The empirical results show: there is no significant difference in the egg's product value (nutrition and freshness) between brand eggs and common eggs, and most consumers are little concerned about the ecological value of eggs. However, there exists significant differences in the safety value (egg's quality) and hedonic value (egg's taste) between these two type eggs, and the higher the value of quality or taste consumers believe, the higher the willingness of their purchase brand eggs. Moreover, consumer's level of income, education and the understanding of egg production also significantly affect their choice of brand eggs positively. These results are useful to decision-makers of egg's enterprises in improving more efficient market strategies, just like promoting the market segmentation and adopting effective measures to guide consumption and enhance China's brand egg market share.

Keywords: brand eggs, market share, extension of the utility maximization model, safety and hedonic value of eggs

S15-0009 Performance assessment of broiler flocks through management and production sensitive Broiler Performance Index (BPI)

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Comparisons of performance between broiler flocks are ineffective when individual criteria such as feed conversion ratio (FCR) or final body weight are compared. The significance of the composite effect of individual performance parameters is unclear to farmers. A survey was conducted to identify the range for individual performance parameters in large, medium and small scale farms (n=160) and feed conversion ratio, survival rate, age at disposal, average live weight at disposal were established. A simple formula for evaluation of overall performance was proposed based on the range of performance parameters. The four significant phenomena have been put together to formulate simple formula to assess the efficiency of the management and production. The following formula was proposed to calculate Broiler performance index considering the relative significance of the each parameter.
$$BPI = ((\text{Live weight (kg/bird)} \times \text{survival rate (\%)} \times 50) / (\text{Age (days)} \times \text{Feed conversion ratio})) - 100$$
 The performance figures which are higher the better were taken as numerators and figures that are smaller the better were taken as denominator. Factors were employed to keep the index within a sizeable values, preferably 0 to 100. BPI at the optimum performance levels of four selected parameters was 41.4 (if FCR= 1.6, weight= 2kg, age =42days and survival =95%). The best and lowest recorded BPI values were 94.5 and - 20.5 respectively for some extreme cases. The proposed BPI was very sensitive to FCR, body weight and less sensitive to age at disposal and survival rate of the flock. BPI is sensitive and positively correlated with the profit margin of the flock. Higher BPI values in a batch compared to a lower BPI in another batch of birds indicates higher profit for the first if the unit price of inputs and produces are equal. The proposed BPI can be used to evaluate and compare performance of broilers flocks.

Keywords: broiler, performance, feed conversion, survival, live weight, profit, analysis

S15-0010 Short and semi-long term evolutions of broiler production in 8 European countries

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The aim of this study was to assess short and semi-long term evolutions of different performance parameters in broilers across Europe. Historical data were obtained from 8 European countries for 2011, 2012 and 2013. Broiler-data were gathered via integrators, individual farmers and governmental organizations. A dataset from at least 50 flocks per country had to be available in a sufficient level of detail. Parameters included: average weight at slaughter, average age at slaughter, average daily gain (ADG); feed conversion rate (FCR), European production index (EPI), overall mortality and condemnation rate. Data-analysis included descriptive analysis and linear mixed modelling, using IBM SPSS statistics 23[®]. Evolutions over time were analyzed at the “Quarter” (short) – and “Year” (semi-long) level based on the date of placement into the broiler-house. In total, 16,422 flocks were included in this analysis, representing over 500 million broilers. The mean (min.-max.) values of the different parameters of dataset level were: 2.46 kg (2.14-2.88) for average weight; 40.14 days (34.98-44.07) for average age; 60.67 gr (57.45-63.44) for ADG; 1.75 (1.64-1.84) for FCR; 337 (303-362) for EPI; 4.11% (3.21-6.18) for overall mortality and 1.15% (0.17-1.92) for condemnation rate. Short term effects revealed some variation between datasets. EPI was up to 4% lower during the first and second quarter of the year in most datasets. Performance related parameters (EPI and FCR) improved significantly over the successive years. EPI improved from 332 (300-355) to 342 (305-371). FCR improved from 1.76 (1.65-1.88) towards 1.70 (1.61-1.83). Clear differences in performance parameters and short term evolutions were found. These variations might be caused by differences in management and climate. The semi-long term effect showed a clear improvement of performance related parameters, EPI and FCR. This work was conducted under the EU-funded PROHEALTH project.

Keywords: broiler-production, European, performance

S15-0011 Short and semi-long term evolutions of broiler-breeder production in 7 European countries

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Efficient performance of broiler-breeder flocks is important to ensure maximum profit. This study aimed to assess short and semi-long term evolutions of different performance parameters in broiler-breeders across Europe. Data were obtained from 7 European countries for 2011, 2012 and 2013 via integrators, veterinarians and governmental organizations. Only datasets with reliable and sufficiently detailed information and with at least 50 flocks per country were included. Parameters included: percentage and age (weeks) at peak of lay, overall mortality of hens (%) and total number of hatching eggs per hen. Evolutions over time were analyzed at the “Quarter” (short) – and “Year” (semi-long) level based on the date of placement into the broiler-breeder house (IBM SPSS statistics 23[®]). In total, 1,427 flocks were included, representing over 18 million broiler-breeders. The mean (min.-max.) values of the different parameters of dataset level were: peak of lay 86.19% (82.92-88.38); age at peak of lay 30.60 weeks (29.71-33.44); overall mortality of hens 8.46% (5.13-12.07); productive period 39.29 weeks (36.57-40.76) and hatching eggs per hen 165.39 (140.15-183.96). Short term: A significant higher peak production of lay was found for flocks placed into the broiler-breeder house during first quarter in comparison with the rest of the year. Semi-long term: Total number of hatching eggs per hen improved significantly from 162.87 (137.77-183.19) in 2011 towards 168.30 (144.36-184.29) in 2013. No clear evolution was present for the other performance related parameters. Clear differences in performance parameters and short term evolution for peak production of lay were found. These variations might be caused by differences in management and climate. The semi-long term effect showed a clear increase of the total number of hatching eggs per hen with 3.23% during the three year period. This work was conducted under the EU-funded PROHEALTH project.

Keywords: broiler breeder-production, European, performance

S15-0013 Economic optimization of broiler slaughter weight and age

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Due to instability of broiler price in Iran, a modeling approach was used to economically optimize slaughter weight and age of broilers according to broiler live price, feed price and fixed costs. A nonlinear (quadratic) relationship between feed intake and weight gain was used in Zanjan broiler farms. A questionnaire based on literature review and experts views, was used and distributed among 102 poultry farmers. The results indicated that feed was the main input in poultry farms and had greatest impact on broiler production. Mean observed feed consumption in Zanjan broiler farms was 5.38 and varied between 3.58-6.8. Mean observed age and weight of slaughter were 51 days and 2.85 kilograms. According to our developed model, estimation of optimum slaughter weight was 2 to 3.3 kilograms in the range of 0.85 to 1.5 US \$ broiler prices. Optimum economic slaughter age of broilers was predicted to be 39 to 55 days in the above mentioned prices range. In conclusion, modeling is a powerful way to optimize broiler production and can be used with high accuracy.

Keywords: broiler, economic optimization, slaughter age and weight, profit

S15-0014 Economics of contract versus independent broiler production in peri-urban bangalore, India

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Contract Farming (CF) can be defined as an agreement between farmers and processing and/or marketing firms for the production and supply of agricultural products under forward agreements, frequently at pre-determined prices. The arrangement also invariably involves the purchaser in providing a degree of production support like supply of inputs and technical advice. In Karnataka, more than 80 per cent broiler production is under CF. Bangalore, being a major consumption centre, CF is also concentrated in the peri-urban Bangalore making it a major production hub. The per farm earnings at constant prices (2005-06 = 100) is the highest among large contract farm category (INR 6,48,181), followed by independent farms (INR 4,23,048), medium contract farms (INR 4,09,466), and small contract farms (INR 1,91,353), in that order. The large contract farms realized a sum of INR 77,939 as growing charges which resulted in earning INR 2.43 per kg of bird lifted. Similarly, medium farms have realized INR 36,170 revenue by way of growing charges, which amounts to INR 2.41 per kg of bird lifted and small CF contract farms from 3473 birds realized INR 16,830 which amounts to INR 2.24 per kilo of bird marketed. The significance of per kg profits of poultry production is tested using one way ANOVA. The significant F statistic indicates that the mean differences between independent and all the three contract producer groups were higher than the respective critical differences. Thus, the performance of broilers is significantly different between contract and independent farms. Resource use efficiency with the help of Cobb-Douglas production function showed elasticity with respect to flock size (0.796) was significant ($p < 0.05$) among contract farms. Similarly on independent farms flock size (0.877) and number of years of poultry experience (0.103) were influencing per farm income along with intangible management factors as revealed by a significant intercept.

Keywords: contract farming (CF); peri-urban; integrators; grower charges; flock size

S15- 0015 Co-integration and price instability of major wholesale egg markets in India

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Engle-Granger Co-integration (EGC) test procedure was applied to examine the performance of wholesale egg markets vis-à-vis their co-integration (oneness). Monthly average wholesale egg price series for the major wholesale egg markets in India, namely, Nammakal, Calcutta, Mumbai, Delhi and Hyderabad for the period 1982 to 2015 were subjected to the co-integration test. First, Augmented Dicky Fuller (ADF) test was applied to the price data (with intercept and trend terms for level and first difference series) for testing presence of unit roots. Having established the condition of non-stationarity and integrating relationships of the same order (i.e., one in this case) for individual price series, the test of co-integration was applied to the residual series obtained in a bivariate scheme (taking level price series in one market as dependent variable and level price series in another market as explanatory variable) using ADF test procedure. The results revealed that these markets were highly co-integrated and hence, they were competitive and efficient at the wholesale levels. The price instability in the selected egg markets was also measured in terms of coefficient of variation (CV) in monthly price data for the split periods 1982-90 and 1991-2015, denoting the periods of pre and post economic reforms. The instability in egg prices increased over time as reflected by increase in CV during 1991-2015 over 1982-90 across all selected markets, which might be due to increased sensitivity and responsiveness of the markets to the dynamic economic forces determining prices of eggs. However, it remained to be examined through primary surveys whether the poultry farmers and traders at the grass-root levels in the country were able to realize prices in accordance with movements in the wholesale market prices of eggs.

Keywords: co-integration, egg, markets, price, instability

S15- 0016 Investigation on the consumption of eggs and egg products in Shandong province

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Shandong province is one of the largest provinces in eggs production and consumption in China. Recently, the consumer expenditure of urban and rural residents on eggs and egg products has been increased steadily. To investigate eggs and egg products consumption characteristics and its influence factor, we conducted a questionnaire survey that focused on places for buying eggs, purchase frequency, concerned factor, price, degree of acceptance of clean eggs, egg product awareness and preference category in the cities of Qingdao, Jinan, Weifang, Linyi and Liaocheng (representing different economic development levels) to provide the basis for better egg production, processing and distribution. The results showed that supermarket and farmer's market were the main places for buying eggs in these cities, accounting for more than 60%, the proportion of purchase frequency every 2-4 weeks wasn't lower than 57%, the most concerned factor was quality and safety problems, representing higher than 82%, the price at 5-8 yuan/500g was accepted by more than 53% consumers, cake was the most preference category for more than 49% consumers. In the cities of Qingdao, Jinan, Weifang, Linyi and Liaocheng, the degree of acceptance of clean eggs for consumers were 30%、27%、21%、11% and 15%, while egg product awareness were 62%、58%、41%、29% and 23% respectively, with differences being both significant ($P < 0.05$), indicating that the economic development level could have huge influence on the consumption of eggs and egg products. It was suggested that the egg distribution channel should be increased, buying less and more frequently advocated. Meanwhile, it was necessary to strengthen the quality and safety management, reduce production costs of eggs and increase yield of clean eggs. Moreover, we should accelerate development of egg products to meet market demand as well as improve the economic development level of developing cities to narrow the consumption gap between different cities.

Keywords: egg, egg product, consumption, clean egg, suggestion

S15- 0018 Innovations and efficiency –strategical goals for development of poultry production in Russian Federation

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Current poultry production in Russian Federation is based on big scale industrial-type enterprises. After the crisis of 1991-1998 the production volumes and per capita consumption have been restored and steadily grow up. In 2015 poultry meat production (slaughtered weight) reached 4425 thousand ton, per capita meat consumption- 30,5 kg. For 2020 these parameters are expected as 4900 and 31,5. Egg production in 2015 was 42,5 bln eggs, per capita consumption- 295 pcs, for 2020- respectively 45 bln and 308 pcs. Russia has practically reached self-sufficiency in poultry products (except pedigree eggs). Now actively grows export potential of Russian poultry industry, for the first time to Asian and African markets. The government will support export efforts of domestic producers. Further development will be related to improvement of quality parameters and economic efficiency- production of high quality, enriched by valuable nutrients poultry products, increased assortment, extended levels of processing, more efficient utilization of feedstuffs, energy, improvement of environment conditions and biosecurity. Very actual task- establishing of domestic breeding centers for layers, broilers, turkeys and ducks, increasing of levels of self-supply in all stages of production. A lot attention and efforts are concentrated on introduction of newest energy, feed and product saving technologies in housing, feeding, processing, storage and marketing. In spite of temporary financial problems there are under way numerous large scale projects in poultry sector. The share of poultry meat in meat consumption balance of Russian population steadily increases. Consumption of shelled eggs has reached reasonable level and there grow the share of egg processing. The role of science in further progress of poultry progress is very important. Now under way is the structural reorganization of scientific institutes in Russia to increase their efficiency.

Keywords: poultry production, innovations, efficiency, development, export, Russian Federation

S15-0019 Poultry meat consumption as an indicator of a better living standard in Europe

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The income elasticity is an economic measure that shows a change in goods consumption as a result of changes in consumer income. The general rule is that a better living standard with higher income increases consumption of normal goods while simultaneously reduces the consumption of inferior goods such as existential foodstuffs. Engel's law establishes that as income increases, households' demand for food decreases proportionally. On the other hand there are highly valued foodstuffs whose consumption does not decrease despite the income growth. The intention of the paper is to determine, at the level of European countries, the correlation between the consumption of poultry meat and GDP per capita. In the EU the average consumption of poultry meat, in 2015, amounted to 22.0 kg per capita. According to the meat type, consumption of poultry meat lags behind of pork (31.0 kg per capita) but is superior to beef consumption (10.1 kg per capita). However, indicative is a trend presenting the growth rate of poultry meat consumption significantly higher than in case of other meat types. In the period 1995-2010, consumption of pork has increased by 0.7%, while the consumption of beef fell by 8.8%. At the same time consumption of poultry meat has increased by 24.7%. Considering the 94% growth of EU GDP, it is clear that the income elasticity of poultry meat is positive and amounts to 0.26 which classified poultry meat as a normal to necessity good ($0 < Ed < 1$). Results show that it is possible to determine a statistically significant difference (Pearson coefficient 0.411, $p < 0.05$) in per capita poultry meat consumption in European countries with different economic situation. Although consumption of all meat types depends on a number of socio-cultural factors, based on positive correlation with GDP per capita, poultry meat consumption can be a valuable indicator of living standard and economic development.

Keywords: income elasticity, poultry meat, GDP per capita

S15- 0020 Influence of agricultural environmental protection laws upon the waste treatment participation will of farmers dealing with large- scale breeding of livestock and poultry: taking an example of layers

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Taking layers as an example, this paper, under Regulations - the agricultural environmental protection law, obtained through research and survey 968 samples of farmers dealing with large- scale layers breeding covering 8 major egg-producing provinces. Plus, it used Heckman correction, a two-stage method which can avoid selection bias of samples, to analyze the influence of agricultural environmental protection laws upon the waste treatment participation will of farmers dealing with large- scale breeding of livestock and poultry. The result shows that: (1) Farmers dealing with large- scale layers breeding maintained that the pollution caused by them is not serious, for excrements are the main source of pollution; only one third of farmers showed the will of participation into pollution control, with each one investing 7600 yuan for it. (2) As an environmental protection law, the Regulations has yet to be known by roughly half of the farmers dealing with large- scale layers breeding since its implementation. Their little knowledge of the Regulations mirrors an insufficiency in disseminating work and cognition degree among them. (3) The Regulations has a significantly positive influence on waste treatment participation will of farmers dealing with large-scale layers breeding, i.e., the more familiar they are with the environmental protection law (the Regulations), the more disposed they get to partake in the pollution control, and the more money they'll invest. This effectively improved the environmental awareness and pollution control awareness among farmers dealing with large-scale layers breeding, and therefore it is reasonable to further promote the disseminating and implementation of environmental protection laws. And (4) the waste treatment participation will of farmers is also positively influenced by their education degree, income, breeding scale and breeding training, as well as the pollution degree and the utilization of excrements.

Keywords: agricultural environmental protection laws, large-scale breeding, waste treatment participation will

S16- 0001 Capacity building: an effective tool for augmenting poultry production a case study of District Hamirpur, Himachal Pradesh

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Hamirpur is the smallest district of Himachal Pradesh wherein the entire poultry population as per livestock census 2007 was a meager 4488 birds. Day old chicks are being supplied by Department of Animal Husbandry to farmers under different schemes but due to insufficient knowledge of poultry husbandry, there was heavy mortality in chicks at the farmer's level. Hence vocational trainings of six days to potential poultry farmers were organized by Krishi Vigyan Kendra Hamirpur. As a result the poultry population in district increased both in backyard and farm/hatchery segment. Poultry population has revealed a growing trend in most of districts and state but there is no parallel to the sharp increase reported in District Hamirpur. It may be attributed to the capacity building programmes conducted by KVK Hamirpur. A momentum in favour of poultry rearing was evident in the period from 2007 to 2012 but how long this momentum is sustained if at all, only time will tell. Capacity building of farmers is an effective tool to augment poultry production and such training should be organized at regular interval to sustain the enterprise.

Keywords: training, KVK, poultry, population

S16-0002 How protective knowledge influence Chinese poultry farmers' intention to adopt personal protective behaviors against A/H7N9

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Poultry farmers are at high risk for infection by Avian Influenza A (H7N9), due to their occupational exposure to live poultry, and therefore need to protect themselves against such exposure. Knowledge is a precondition to change poultry farmers' personal protective behaviors (PPBs). To significantly improve the PPBs among poultry farmers, we should figure out the mechanism how protective knowledge influence poultry farmers' intention to adopt PPBs. The survey (n=297) was conducted using questionnaire to measure the level of awareness of knowledge items related to A/H7N9, and the threat appraisal, coping appraisal to the A/H7N9 epidemic situation among poultry farmers in Chinese Jiangsu Province. Exploratory Factor Analysis revealed four main types of knowledge, including Knowledge of symptoms, Knowledge of measures for self-protection during work, Basic preventative knowledge, and Routine hygiene knowledge. Structural Equation Model (SEM) indicated that Knowledge of symptoms and Knowledge of measures for self-protection during work, as well as the Basic preventative knowledge are significant determinants of coping appraisal (the self-efficacy and response efficacy). The moderate perceived response efficacy of respondents and its strongest association with protective intention reflected that intervention by propagandizing the knowledge of symptom and working protection, as well as the basic protective knowledge to enhance perceived response efficacy may effectively motivate adoption PPBs.

Keywords: Chinese poultry farmers, A/H7N9 epidemic, protective knowledge, intention to adopt personal protective behaviors, structural equation model

S16-0003 Assessment of the knowledge and marketing of poultry of small family farms

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This paper concentrate on revealing the significance of small family farm by community in south west Nigeria. A survey was conducted on determining the general knowledge of the farmers on diseases and marketing structure of poultry production in small family level in south west, Nigeria. This was done through communication with 62 persons via questionnaire. In some cases lack of accounting records led us to use a face to face survey method in the study. Elements of disease and market structure were determined. Percentage and mean values of the data were calculated using SPSS version 11.0. (The statistical package for the social sciences) package program. Student t-test was used to reveal significance of the differences between the groups, using confidence limits of $P < 0.05$. The findings revealed that the majority (64.7%) of the poultry farmers were male and only keep between 50-100 birds which depicts that the respondents are small scale farmers, however 67.7% of respondents make use of family labor. The study revealed that small business on poultry at family level typically are not profitable, but because women and children are mostly in charge of taking care of the farm, demanding no wages in return therefore the labor cost is low. Sources of capital is a problem to most of the farmers, only 31.9% were able to receive loans from banks and cooperatives. The results also revealed outbreak of diseases in some farms. It is unfortunate that even though 90.3% of farmers have heard of Avian influenza, many of them did not have the knowledge of the symptoms, this show that there is a wide gap in awareness and management of this disease. Therefore the need for vocational training and use of information technology cannot be over-emphasized. A fairly large proportion have ready market for their birds while others sell through middle men. The correlation analysis shows that the amount realized from the sales of birds by the farmers was not affected by their location and education.

Keywords: disease, profit, family, market, training

S16- 0004 Knowledge empowerment through distance education

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Agriculture including Animal Husbandry is the primary avocation for 70 per cent of the population in India. Indian animal husbandry practices have been contributing nearly 30 per cent of the gross domestic product in agriculture. In the changing scenario, farming enterprises like dairying, poultry, fisheries and allied sectors etc., are transforming into industrial ventures in which there exists a great demand for skilled and trained manpower on various animal husbandry techniques in the private sector. Moreover technological interventions are available at ease in the commercial farms whereas landless labourers, small and marginal farmers encounter lot of constraints while undertaking farming activities. Under these circumstances, Directorate of Distance Education, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, India has launched skill development training courses namely poultry farm manager, poultry farm supervisor, poultry breeder farm supervisor, hatchery supervisor, poultry vaccinator and turkey farm assistant and self-employment training courses namely desi- chicken farming and Japanese quail farming for the benefit of various categories of the people. The skill development and self-employment courses are being offered through four veterinary colleges, eight research stations and 28 extension centres of TANUVAS. These courses have 1-3 months duration with minimum qualification of reading and writing in vernacular language up to higher secondary education. The course curriculum was designed in such a way that practical aspects in poultry farming have been given importance. The participants have gained knowledge and skill in poultry sector for employment in corporate poultry sector and to run poultry farm on their own.

Keywords: skill development, self employment, poultry, courses

S16- 0005 Vocational education and training in poultry production in Kingdom of Saudi Arabia

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The biggest poultry producers in Saudi Arabia are rapidly increasing their production capacities to cope with the nation's demand for poultry meat which is further stimulated by the strong annual population growth rate (2.1% in 2014) and the per capita poultry meat consumption (47 kg per year). Considering the ever-increasing demands for poultry meat and eggs, the poultry industry of Saudi Arabia has a great need for highly skilled employees. AL Watania Poultry Institute of Technology (WIT) has been established as a strategic partnership between the largest integrated poultry company in the Middle East, Watania Poultry Co (WPC) and the government of Saudi Arabia, represented by Technical & Vocational Training Corporation (TVTC). This new entity WIT, aims to provide world-class education and practical training for Saudi workforce in the field of poultry industries in order to assist them in securing employment in poultry private sector. WIT has developed educational programs of highest quality and relevance to poultry industry by methodically analyzing the job descriptions and competencies needed in daily routine jobs of WPC. The developed curricula are based on intense English and Basic sciences courses, including introductory lectures in poultry science, during the first foundation year and on entirely Poultry Management courses and On-Job Training during the rest of the studies period. State-of-the-art laboratories and newly built research and training facilities were established and equipped with the latest technologically advanced equipment so that deep practical training in all aspects of production can be offered. The research facilities are fully environment controlled and able to accommodate statistically significant number of birds and to replicate any experimental protocols. In conclusion, the combination of infrastructure with highly experienced teaching staff ensures that WIT will deliver highly skilled trainees for the needs of poultry industry in KSA.

Keywords: vocational education, poultry production, Saudi Arabia

S16- 0006 Sasso colored broiler– a low external inputs bird for rural India

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Livestock Extension is important for convincing the farmers while introducing a new strain or variety of birds. The present field level performance trial on SASSO colored broilers was supervised as part of Rural Livestock extension work by the Dept. of Animal Sciences, University of Agricultural Sciences, Bangalore with the help of a group of progressive farmers in South India during 2013. One complete cycle of 8 weeks duration was studied to find out suitability of this breed as a low input technology bird for rural farmers under existing agro climatic conditions and locally available inputs. This performance study is based on 1000 SASSO day old chicks supplied by Ayilas Farms Hatchery, Coimbatore and chicks arrived at the farm after travelling for 30 hours by Train as well as Road. Charcoal brooders, drinkers and feeders in an open sided shed on deep litter system and locally available commercial broiler feed and vaccines were provided. Quality water was taken from the farm bore well. ND live vaccinations were done at week 1 and 4 and IBD live at week 2. Mortality and feed quantity were recorded on daily basis, body weights on 10% random birds on weekly basis and feather colors at week 6. Birds gained 1 kg live weight by 40th day with 1.88 FCR and 1.5 kg by 56 days age with 2.2 FCR. Mortality was 2% , mostly during first week. There was no aggressive behavior like pecking. Clear color pattern was seen by week 3; multi-color feather pattern with mixture of brown, black, red, golden, grey and a few with Sussex pattern but no white birds were noticed. Total cost of production was INR 80 per kg and birds were sold live at INR 100 per kg locally resulting in a profit margin of INR 30 per bird sold. Customers reported excellent flavor, taste and texture of the meat, close to that of country chicken. Thus, SASSO colored broilers were found to be suitable for rural poultry production which accounts for 15-20% of market size in India.

Keywords: live stock extension, low input technology, innovators, charcoal brooder, feed conversion ratio (FCR)

S16- 0007 Peer to peer extension: flock talk forums

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Often valuable learning opportunities happen organically after a formal lecture; e.g. discussions of participants over dinner or in the hallways solidify or clarify what was presented more formally earlier. E.M. Rogers (2003) demonstrated that peer to peer learning is an effective tool for research adoption. Utilizing a variety of extension approaches will increase the likelihood of adoption of new management techniques. A peer-to-peer education opportunity was developed that brought together 28 primary poultry producers and 4 subject matter experts for an informal forum on nutrition. The event was held in a semi-private room at a restaurant and refreshments were served throughout the event. Handouts were provided to each producer that introduced each expert and included suggestions of topics they were prepared to address, along with space for notes. Producers were not limited to these topics. Producers were grouped by industry (egg, turkey or broiler chicken) to increase the opportunity for producers to learn from each other based on their personal experiences. The experts served as table hosts for a half hour discussion; after which the expert rotated to the next group of producers. Every producer group had the opportunity to have a discussion with each expert. Group size was limited to a maximum of 8 including the expert, based on E. Hall's (1976) conclusion on effective group sizes. Ground rules were established that allowed all participants to speak and be heard. Of the 28 producer participants, 100% rated the event as good to excellent. 75% indicated that the event format helped them learn a variety of topics quickly and easily; 64% responded that the format allowed them to ask questions they had been curious about. 57% planned to try something new they learned at the event. Producers valued the time with the experts and the opportunity to learn from fellow producers. Informal discussion forums appear to be a viable peer-to-peer extension approach.

Keywords: peer to peer learning, discussion forum, research adoption

S16-0008 Future challenges for profitability of backyard poultry in Pakistan

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Poultry birds which are kept in houses for the purpose of meat, eggs and as pets are known as backyard poultry and it also covers the fancy birds. 80% of poultry flocks in the world are backyard poultry flocks and flock size ranges from 3-5 birds to few 100 birds of different ages and breeds. Our indigenous poultry possesses bright future development due to abundant availability of all the requisites and minimum cost of production. However, production of these birds is very low due to various factors like imbalanced feeding and housing, lack of proper vaccination and biosecurity, mixing of multi-age birds, close contact with wild birds and different diseases. According to the National Animal Health Monitoring System Poultry (NAHMSP) survey the most common health complications of backyard poultry are external parasitic problems, respiratory illnesses and large number of unidentified deaths. Most farmers are inexperienced and due to small flock size they don't bother about treatment and another reason is no/few diagnostic laboratories working far away from the villages. Another constraint is predation by wild animals. In short to make the backyard poultry more profitable there is dire need to improve local breeds with high production potential due to the advantage of adaptability to the local environmental conditions and as a result rearing cost can be reduced. Education/awareness of the farmers regarding health, feeding and management is necessary along with establishment of diagnostic laboratories at village levels.

Keywords: poultry, backyard, challenges

S16-0009 Value chain in broiler contract farming and business performance in West Sumatra Indonesia

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Broiler business partnerships with contract farming have necessary role to increase the production of broilers in the West Sumatra. The objectives of this research are (1) to map the value chain of broiler industry at Japfa Comfeed Indonesia (JCI) in Padang city, Indonesia; (2) to analyze the value chain governance of broiler industry at JCI; (3) analyze the margin of marketing at broiler industry both on farm or off farm; (4) to analyze the possibility of the construction of modern chicken slaughterhouse to cope with excess production of broiler chicken meat. Value chain analysis has been widely used in agriculture to identify necessary role and intervention for a better situation of agriculture and rural development in Indonesia. Findings of this research show that the role of JCI in this value chain is highly significant. All inputs including DOC, feed, veterinary medicine, and poultry equipments are supplied by JCI. However, for downstream sector, JCI acts as product distributor. The type of value chain governance among suppliers of feed, DOC, veterinary medicine, poultry equipment, and poultry derivatives belongs to market value chain. Whereas the type of value chain governance between broiler supplier and restaurant belongs to captive value chain. Based on the margin of marketing analysis, the party that gains the lowest margin is farmer and the party that receives the highest margin is broiler distributor. This finding reflects great opportunities to establish a chicken slaughterhouse.

Keywords: value chain, margin, excess production, chicken slaughterhouse

S16-0010 “Kamrupa” –A dual type new variety of chicken for rural poultry production in Assam and North East India

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The people of Assam and North East India generally rear some indigenous bird to meet up their requirement of egg and meat. Kamrupa is a newly developed multicolour dual type rural variety. The present investigation is to evaluate the comparative performance of Kamrupa and Indigenous chicken for suggesting the better variety for rural poultry production. For the proposed study 2000 nos of chick were hatched out and brooded in battery brooder for a period of 30 days. Thereafter the chicks were distributed to 50 farmers to be reared in scavenging condition with a allocation of 20 chicks from each group. The birds had been supplied with some quantity of rice polish and broken rice apart from all the feed they eaten during scavenging. Routine managerial practices had been followed throughout the period of study. The traits recorded were body weight, conformation traits, average age at maturity, egg production, egg weight, egg quality and carcass traits. To study egg quality and carcass traits 300 eggs and 80 adult chickens of 10 months old were taken. The data were analyzed as per the method described by Snedecor and Cochran (1994). The results obtained are recorded as body weight (g) from day old to 40 weeks which ranges from 29.65 ± 2.12 to 1825.38 ± 141.56 in both the flock. Among the conformation traits the shank length, keel length and breast angle ranges from 3.89 ± 0.76 cm to 63.15 ± 2.64 degree. The average age at sexual maturity, egg production upto 32 and 40 weeks, annual egg production and egg weight at 32 weeks and 40 weeks of age ranged from 21.72 ± 1.25 nos to 210.68 ± 3.47 days. Among the egg quality traits shape index, albumin index, yolk index, Haugh unit and shell thickness were ranged from 0.079 ± 0.001 to 79.24 ± 1.56 . The values for different carcass traits ranges from $63.69 \pm 2.89\%$ to 1931.20 ± 182.28 g. Performance of Kamrupa bird is found better than that of Indigenous chicken

Keywords: Kamrupa bird, performance traits, extensive system, indigenous bird and egg and carcass traits

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