DETERMINATION OF ZEARALENONE AND ITS METABOLITES IN BOVINE FEEDING STUFF AND ASSESSMENT RESIDUES IN BOVINE URINE.

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Introduction

Zearalenone (ZON) is a mycotoxin that contains a nonsteroidal resorcyclic acid lactone and it is produced mainly by several species of *Fusarium* fungi which grown in cereal grains (eg. *F. culmorum and F. graminearum*). If grains contaminated with ZON are used for animal feed, it is considered to be potentially hazardous to animals.

Furthermore, ZON possesses many characteristics similar to those of steroid hormones and binds to both types of estrogen receptors. ZON can be metabolized to at least five metabolites including α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), zearalanone (ZAN), α-zearalanol or zeranol (α-ZAL) and β-zearalanol or taleranol (β-ZAL). α-ZAL and β-ZAL possess estrogenic activity and have been shown to cause hyperestrogenic and adverse metabolic effect in livestock and poultry. Because of its anabolic effect, α-ZAL had been used as a growth promoter. Although its application has been banned in the European Union [Commission EC,1996], there is still a need to monitor its presence. So that, we present a study with two objectives, one analyce the presence of ZON and its metabolites in bovine feed and second to analyce their presence in urine of animal which have consumed this feed.

Materials and Methods

Chemicals and instrument

ZON, α-ZAL and β-ZAL were purchased from Sigma® and as internal standards (IS) zeranol-D4 was used and was purchased from RIVM. Organic solvents used were acetic

acid from Scharlau®; methanol and acetonitrile supplied from Merck®; and hydrochloric acid, sodium chloride and ammonium hydroxide from Panreac®. All solvents used in sample preparation were HPLC grade and chromatographic separations LC-MS-MS grade. Extraction cartridges C_{18} (500mg/3mL) and Bond Elut Plexa™ (500mg/3mL) were purchased from Waters® Co. and Varian®, respectively.

Chromatographic analysis of the analytes was carried out on a Finnigan® Surveyor Autosampler coupled to a Finnigan® Surveyor LC Pump and to a ThermoFinnigan® TSQ Quantum Ultra AM triple quadrupole detector (Milford MA, USA). The analytical column was a Hypurity C_{18} (2,00 x 150 mm, 3 µm) in combination with a guard column with the same characteristics as Hypurity C_{18}, and were purchased from Thermo®.

Sampling

Feed and urine samples were purchased in farms from Valenciana Community.

Extraction procedures

a) Feed samples

Feed samples were milled and homogenized and next, 2.5 g of homogenized sample was weighed in a plastic tube, followed by addition of 4 mL of methanol and 4 mL of acetic acetate 2 M (pH: 4.8). After vigorously shaking, the tube was placed in centrifugation at 4.000 rpm for 10 min.

The supernatant were transferred to a cartridges of C18 Bond Elut previously conditioned with 4 mL of water at 10% of sodium carbonate, 4 mL of water and finally 4 mL of sodium acetate 2 M (pH: 4.7). After washing the cartridge with 4mL of water and 4 mL of methanol:water (50:50, v:v) it was dried for 2 min applying vacuum. To elute the analytes, 4 mL of methanol was used. The elute was evaporated to dryness under a stream of nitrogen and 45 ºC. The residue was reconstituted in 500 µL of the LC-MS/MS mobile phase, acetonitrile:water (30:70) 0.1% acetic acid, and 25 µL of this solution was injected into the LC-MS/MS system.

b) *Urine samples*

Bovine urine (5 mL) was mixed with 1 ml of buffer acetic/acetate (pH: 4.8) and 5 µL of 1µg/ml D2-ZAN. This solution was incubated for 3h at 37 ºC with 10 µL of β-glucuronidase/arylsulfatase solution. After the tube was placed in centrifugation at 4.000 rpm for 10 min.

The supernatant were transferred to a cartridges of C18 previously conditioned with 4 mL of methanol and 4 mL of water. After washing the cartridge with 4mL of

methanol:water (45:55, v:v) it was dried for 2 min applying vacuum. To elute the analytes, 4mL of methanol was used. The elute was evaporated to dryness under a stream of nitrogen and 45 ºC. The residue was reconstituted in 500 µL of the LC-MS/MS mobile phase, acetonitrile:water (30:70) 0.1% acetic acid, and 25 µL of this solution was injected into the LC-MS/MS system.

Results and Discussion

ZON, α-ZAL and β-ZAL were simultaneously detected in bovine feed and bovine urine by a reproducible, sensitive and selective multi-residue analytical method by liquid chromatography tandem mass spectrometry using negative electrospray source (LC-MS/MS). Recoveries were achieved above 82% in urine samples, and more than 79% in bovine feed samples. The decision limit (CCα) was 0.01 µg/L and 22 µg/kg and the detection capability (CCβ) was 0.5 µg/L and 25µg/kg.

Conclusions

A total of 95 animal feed samples and 102 bovine urine samples were analyzed. In animal feed, 59 samples contained ZON and only one β-ZAL. While in analyzed bovine urine 22 samples were contained ZON, 14 with α-ZAL and 3 with β-ZAL.

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References

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