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A Review

Probiotics in man and animals

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1. Introduction

The ready availability of antibiotics in the 1950s resulted in their widespread use as therapeutic agents and growth stimulants for farm animals. Since that time there has been growing concern that the use of antibiotics as growth promoters was resulting in the development of resistant populations of bacteria which made subsequent use of antibiotics for therapy difficult. Their use as animal feed supplements was curtailed by the Swann Committee in 1969, whose recommendations resulted in the restriction of growth-promoting antibiotics to those which were not used in the treatment of disease. Since then the permitted antibiotics and other chemical feed supplements have been widely used. Recently, however, they have come under renewed scrutiny from the 'anti-additive' lobby and some supermarkets are already selling antibiotic-free meat.

There is also a reaction against the use of antibiotics as therapeutic agents because of the intestinal upsets which often follow oral treatment with these agents. Although they are effective in curing the disease for which they are prescribed, the effect on the indigenous gut flora may persist after cessation of the treatment.

The possibility of antibiotics ceasing to be used as growth stimulants for farm animals and the concern about the side-effects of their use as therapeutic agents has produced a climate in which both consumer and manufacturer are looking for alternatives. Probiotics are being considered to fill this role and already some farmers are using them in preference to antibiotics.

2. Definition

Over the years the word probiotic has been used in several different ways. It was originally used to describe substances produced by one protozoan which stimulated another (Lilly & Stillwell 1965) but

was later used to describe animal feed supplements which had a beneficial effect on the host animal by affecting its gut flora (Parker 1974). In its latter role it was defined as 'organisms and substances which contribute to intestinal microbial balance'. This definition is unsatisfactory because it is too imprecise; it would include antibiotics. I have revised the definition to read 'A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance'. This revised definition emphasizes the importance of *live* cells as an essential component of an effective probiotic and removes the confusion created by the use of the word 'substances'.

3. The gut microflora and its contribution to resistance

The foetus *in utero* is sterile, but on passage through the vagina during birth it acquires micro-organisms. These are rapidly added to after birth and the new-born animal acquires a gut microflora which is characteristic for its species. In the wild state the animal obtains its gut flora from its immediate environment which is heavily contaminated with bacteria from the mother.

The final indigenous gut microflora which stabilizes in the gut is a very complex collection of about 10^{14} micro-organisms consisting of 400 different types of bacteria (Moore & Holdemann 1974) many of which are listed in the review by Tannock (1988a). Within such a complex system are many interrelationships between different micro-organisms and between micro-organisms and the host. In spite of all this scope for variability, the flora quickly settles down to a very stable population. The composition of the flora is determined by host and microbial factors (see Fuller 1982; Fuller *et al.* 1986) and although there are a lot of bacteria which can survive and grow in the intestinal tract, there are many which cannot. Not only do the successful ones have to run the gauntlet of the antimicrobial chemicals present in the gut, but they also have to avoid the effects of peristalsis which tends to flush out bacteria with the food. This can be done either by immobilizing themselves by attaching to the gut wall, or by growing at a rate which is faster than the rate of removal by peristalsis. The survival of probiotic organisms in the gut depends on their possessing colonization factors which enable them to resist the antibacterial mechanisms (chemical and physical) which operate in the gut.

The stable flora which develops in the intestine helps the animal to resist infections, particularly in the gastrointestinal tract. The phenomenon has been described by various authors and given the names bacterial antagonism (Freter 1956), bacterial interference (Dubos 1963), barrier effect (Ducluzeau *et al.* 1970), colonization resistance (Van der Waaij *et al.* 1971), and competitive exclusion (Lloyd *et al.* 1977). The best evidence for this protective effect of the gut flora stems from the observation that germ-free animals are more susceptible to disease than are the corresponding conventional animals with a complete intestinal flora. For example, whereas a germ-free mouse can be killed with 10 cells of *Salmonella enteritidis*, it requires 10^6 cells to kill a conventional mouse (Collins & Carter 1978). The presence of a gut flora is the important factor in this difference because the LD_{50} for germ-free and conventional mice is the same if the animals are challenged i.v. or i.p.

Support for this claim comes from the experience of clinicians with antibiotics given *per os*. This practice often induces intestinal infections resulting in enteritis and diarrhoea. In chickens the inclusion in the feed of subtherapeutic levels of antimicrobial growth promoters frequently prolongs the excretion of salmonellas in the faeces (Smith & Tucker 1975) and a similar effect is obtained in mice dosed experimentally with antibiotics (Bohnhoff *et al.* 1954; Freter 1955, 1956). In all these cases the antimicrobials are suppressing the protective flora and allowing the pathogen to survive.

4. Causes of induced changes in the gut flora

The protective flora which establishes itself in the gut is very stable, but it can be influenced by some dietary and environmental factors. The three most important are excessive hygiene, antibiotic therapy and stress.

In the wild state the baby animal picks up its gut flora mainly from its mother by direct or indirect routes (Fig. 1). However, modern methods of animal rearing and baby care often restrict the access that the infant has to the mother and prevents it acquiring the full complement of characteristic microbes. The chicken is a good example of this phenomenon. The egg is removed from the mother

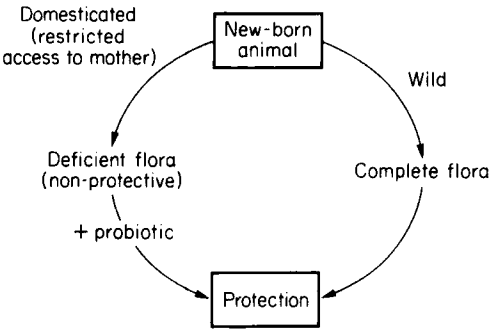

Fig. 1. Source of intestinal micro-organisms in the wild and domesticated young animal.

and hatched in a clean incubator. There is no direct contact with the hen and the chick acquires its flora from the incubator environment.

With mammals the separation is less complete but there is still a tendency, especially with the human baby, to minimize the transfer of bacteria from mother to child by the practice of excessive hygiene and the over-use of disinfectants. Whereas excessive hygiene prevents the acquisition of a protective flora, oral antibiotics suppress its activity even after it has been acquired. Thus diarrhoea is a common side-effect of *per os* antibiotic treatment. The disease pseudomembranous colitis is almost always associated with administration of antibiotics by mouth, and *Candida* infections are often an unpleasant consequence of antibiotic therapy. Antibiotic-treated mice are more easily infected with *Candida* (Huppert *et al.* 1955; Seelig 1966) and *Candida* upgrowth induced by chemotherapy can be restricted by feeding patients with a preparation containing *Lactobacillus acidophilus* (Tomoda *et al.* 1983). Antibiotics can also aid the colonization of the gut by lactobacilli (Bhattacharya & Majunder 1983) and this may be a useful way of preparing the gut to accept probiotic strains.

The antibiotic-sensitive protective effect has been called 'colonization resistance' (van der Waaij *et al.* 1971) and its effect in mice has been ascribed by some workers to strict anaerobes (Van der Waaij *et al.* 1971) and by others to facultative anaerobes (Freter & Abrams 1971). The evidence available for humans indicates that antibiotics, which suppress the anaerobic gut flora, do not reduce colonization resistance (Gorbach *et al.* 1988).

The established protective flora can also be affected by stress (see Tannock 1983). During stress conditions the general trend is for the lactobacilli to decrease and the coliforms to increase. Stress can be produced by drastic changes in the physical or emotional environment. The hormonal changes which ensue can affect the production of mucus which may in turn reduce the components of the gut flora which are usually associated with it. The inevitable stress which accompanies space flight and the preparation for it is associated with a change in the lactic acid bacteria present in the gut (Lizko *et al.* 1984; Lencner *et al.* 1984). Terrestrial travel can also induce changes in the flora which often manifest themselves in diarrhoea.

These conditions, where the balance of the gut microflora is adversely affected, are all situations in which probiotics are of potential value. The restoration of the gut flora will enable the host animal to return to normal.

5. Composition of probiotics

Probiotics can be presented to the animal in various ways. The type of preparation will depend on the sort of use intended. They can either be included in the pelleted feed or produced in the form of capsules, paste, powder or granules which can be used for dosing animals directly or through their food. The target species are cattle, sheep, goats, pigs, poultry, horses and domestic pets.

Nearly all of the probiotics currently on the market contain lactobacilli and/or streptococci; a few contain bifidobacteria. Probiotic preparations may consist of single strains or may contain any

number up to eight strains. The attraction of multiple-strain preparations is that they are active against a wider range of conditions and in a wider range of animal species.

The species currently being used in probiotic preparations are *L. bulgaricus*, *L. acidophilus*, *L. casei*, *L. helveticus*, *L. lactis*, *L. salivarius*, *L. plantarum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Ent. faecalis*, *Bifidobacterium* spp. and *E. coli*. With two exceptions these are all intestinal strains. The two exceptions, *L. bulgaricus* and *Strep. thermophilus*, are yoghurt starter organisms and are included on the basis of the claims made for yoghurt.

The choice of the other lactobacilli and streptococci may also have been influenced by the yoghurt health claims. Yoghurt itself can be regarded as a probiotic, although some of the effects which it has may not be dependent on the presence of live bacteria in the gut. For example, the reduction of *E. coli* seen in pigs can be reproduced by milk acidified with lactic acid (Ratcliffe *et al.* 1986). Similarly in human flora rats reduced coliform counts were obtained by feeding either acidified milk or pasteurized yoghurt (R. Fuller and C.B. Cole, unpublished data). However, the increased lactase activity of the gut, after ingestion of yoghurt, is dependent on microbial enzyme activity and requires the presence of live yoghurt organisms in the intestine (Garvie *et al.* 1984).

Lactobacilli and streptococci are the most commonly used groups in the production of probiotics. The justification for the use of lactobacilli stems from studies which show that when the gut flora develops after birth, as the lactobacilli increase, other components of the flora decrease (Smith 1965). Experiments with gnotobiotic chicks have confirmed that lactobacilli exert a controlling effect on *E. coli* (Fuller 1978). Similar results were obtained in pigs weaned when two days old on to a sow's milk substitute diet. When these pigs were given lactobacilli in their milk there was a significant decrease in the *E. coli* count in the stomach and duodenum (Barrow *et al.*, 1980).

However, the situation is complicated by the finding that some of the strains of so-called *Ent. faecium* used as probiotics are not *Ent. faecium* but an unidentified strain of *Enterococcus* (J. Farrow, personal communication) and the strain which causes growth depression is not *Ent. faecium* but a new species called *Ent. hirae* (Farrow & Collins 1985). It may be that the two similar organisms are competing for the same niche and the beneficial effect is produced by the probiotic strain successfully competing with and eliminating the growth-depressing strain.

Some probiotics contain *Bacillus subtilis* as one of the components. However, it is difficult to see how this can be active in the gut; it is certainly not an intestinal organism and, since it is a strict aerobe, would not be able to grow or metabolize in the gut.

6. Mode of action of probiotics

The beneficial effects of probiotics may be mediated by a direct antagonistic effect against specific groups of organisms, resulting in a decrease in numbers or by an effect on their metabolism or by stimulation of immunity (Table 1). For all these mechanisms there is some support from experimental data. The suppression of bacterial numbers could be produced by production of antibacterial substances. Primary metabolites, such as organic acids and hydrogen peroxide, are known to be effective *in vitro*. However, the evidence for the involvement of organic acids in the control of gut bacteria is

Table 1. Possible modes of action of probiotics

1. Suppression of viable count by:
   (a) production of antibacterial compounds
   (b) competition for nutrients
   (c) competition for adhesion sites
2. Alteration of microbial metabolism
   (a) increased enzyme activity
   (b) decreased enzyme activity
3. Stimulation of immunity
   (a) increased antibody levels
   (b) increased macrophage activity

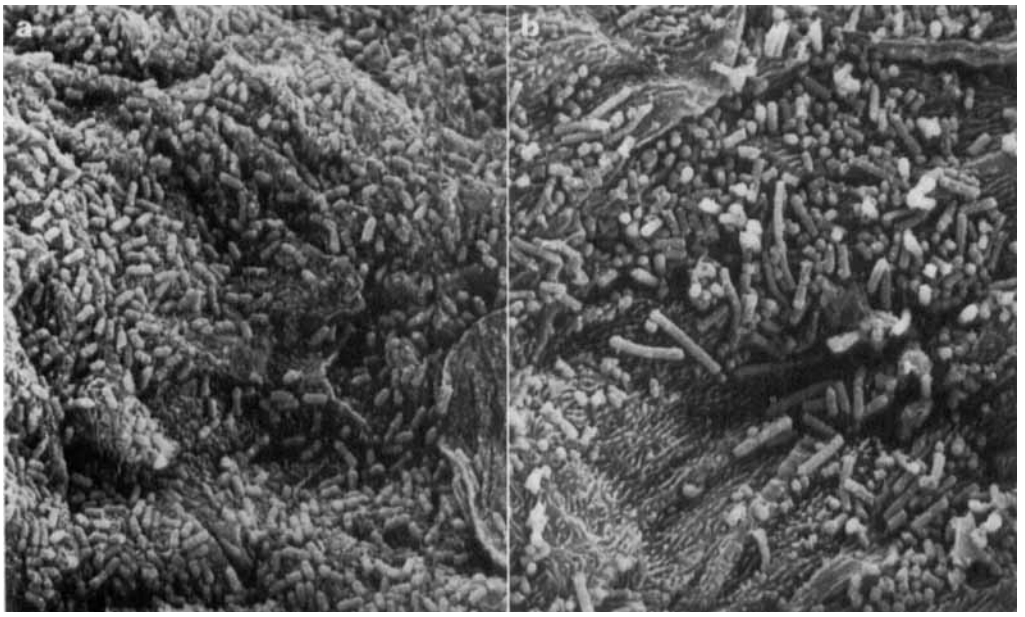

Fig. 2. (a) Scanning electron micrograph of chicken crop wall showing lactobacilli attached to the surface. (b) Scanning electron micrography of the *pars oesophagea* region of the pig stomach showing streptococci and lactobacilli attached to the squamous epithelial cells.

equivocal (see Hentges 1983). Several high molecular weight antibacterial substances have been described as being produced by lactic acid bacteria, but some authors think that the observed inhibitory effects can, in many cases, be accounted for by low pH and primary metabolites (ten Brink *et al.* 1987). None is known to be active in the intestine.

Another mechanism for preventing colonization by pathogens is competition for adhesion sites on the gut epithelial surface. Lactic acid bacteria are known to be associated with the gut wall of chickens (Fuller & Turvey 1971) and pigs (Barrow *et al.* 1980) (Fig. 2) and the competitive exclusion effect in chickens can be produced with material which remains attached to the caecal wall after washing four times in buffered saline (Stavric *et al.* 1987). This sort of evidence suggests that it is desirable to use adhering strains when designing probiotic supplements. However, it should be borne in mind that adhesion is a host specific phenomenon (Fuller 1973; Barrow *et al.* 1980) and that adhesion varies between strains of the same species (Barrow *et al.* 1980) and can be influenced by the growth conditions and media used (Fuller 1975; Conway *et al.* 1987).

The way that lactobacillus supplements can influence microbial metabolism in the gut is demonstrated by the work of Goldin & Gorbach (1984). When they fed *L. acidophilus* to human subjects and looked at selected enzymes, the treatment suppressed the activity of β-glucuronidase, nitroreductase and azoreductase. Similarly, in rats associated with a human gut flora there was a reduction in β-glucuronidase and β-glucosidase when they were dosed with the same strain of *L. acidophilus* (Fuller & Cole, unpublished data). Probiotics may also have their influence by increasing the activity of useful enzymes, e.g. β-galactosidase in the alleviation of lactose intolerance (see below).

Although on theoretical grounds it seems very likely that competition for nutrients will operate in the gut, the evidence for it occurring is not good. In-vitro studies demonstrated a competition for carbon sources between the gut flora and *Shigella flexneri* (Freter 1962) but the antagonistic effect could be changed by altering the medium (Hentges 1983). There was not a good correlation between the antagonistic effect of specific bacteria when tested *in vitro* and *in vivo* in monoassociated mice

(Maier & Hentges 1972). Although other mechanisms cannot be completely ruled out, the competition by unidentified components of the gut flora for specific carbohydrates is at least partially responsible for the suppression of *Clostridium difficile* in the normal mouse caecum (Wilson & Perini 1988).

Conventional animals with a complete gut flora have increased phagocytic activity and immunoglobulin levels compared with germ-free animals (see Bealmear *et al.* 1984). A strain of *Ent. faecium* (a species frequently used in probiotics preparations), established as a monoassociate in germ-free mice, was able to reduce the amounts of *Salm. typhimurium* in the spleen (Roach & Tannock 1980) implying a systemic effect. Yoghurt has been shown to increase antibody levels when fed to germ-free mice (Wade *et al.* 1984) and lactobacilli are also involved in the stimulation of phagocytic activity. *Lactobacilli casei* in particular, was active in this respect when administered *per os* to mice (Perdigon *et al.* 1986). In order for the bacteria to have these kinds of systemic effects it may be necessary for them to migrate from the gut to the systemic circulation. Lactobacilli can translocate (see Berg 1983) and can survive for many days in the spleen, liver and lungs (Bloksma 1981). The results obtained with lactobacilli given parenterally are, therefore, of relevance to our understanding of the probiotic effect. For example, *L. casei* and *L. plantarum* given parenterally stimulate phagocytic activity (Saito *et al.* 1981; Kato *et al.* 1983; Bloksma *et al.* 1981). Natural killer cell activity was also enhanced by *L. plantarum* (Bloksma *et al.* 1981). Lactobacilli can also be used to prevent the growth of tumours (Kato *et al.* 1981; Friend & Shahani 1984; Reddy *et al.* 1983) and their importance in cancer prevention has been suggested, but to date there are no clinical trials to support this. However, these findings of a systemic effect on immunity do indicate that probiotics have the potential, not only to affect the balance of the gut flora, but to influence the pathogenesis of diseases which occur in tissues remote from the intestinal tract.

7. Practical results with probiotics

7.1 GROWTH PROMOTION OF FARM ANIMALS

Probiotics have been used as growth promoters to replace the widely used antibiotic and synthetic chemical feed supplements. However, there are few published reports of good controlled field experiments and the comprehensive assessment of their value has not been attempted in the form of a large scale co-ordinated field trial. The results of probiotic supplementation of chicken diets have been variable but there have been reports of statistical effects on growth (Dilworth & Day 1978) and egg production (Miles *et al.* 1981).

Baird (1977) obtained an increase in daily weight gain and an improvement in feed conversion in separate experiments with feeder pigs and growing-finishing pigs using a lactobacillus supplement. With the same probiotic Pollman *et al.* (1980) obtained a positive result with starters but not with growing-finishing pigs. They suggested that the lack of effect in the older pigs may have been due to the use of a different diet; it was less complex than the diet used for the starter pigs.

When Pollman (1986) summarized the results he obtained with starter pigs over a long period he found that although they were variable, the mean of the pooled data showed a positive effect. For lactobacillus fermentation products, mixed lactobacillus supplements and single-strain lactobacillus supplements, the percentage improvement in weight gains was, respectively, 8·4, 2·5 and 8·6.

Bacteria other than lactobacilli have been used as growth promoters. Han *et al.* (1984a,b,c) studied the effect of supplementing chickens and pigs with an aerobic sporeformer (so-called *Lactobacillus sporogenes*) and *Clostridium butyricum*. The supplements significantly improved weight gain and feed conversion of chickens. In pigs the growth response was not significant but the improvement in feed conversion was. Both the supplements suppressed the counts of staphylococci and coliforms in chickens as well as pigs. Mordenti (1986) found that the growth promoting effect which he obtained by feeding *Ent. faecium* to pigs could be improved synergistically by the addition of whey peptides.

Diet is only one of several factors which may be influencing the results obtained with probiotics. The growth stimulatory effect in itself is bound to be variable. It will operate only when the animals

are stressed by the presence of a growth depressing microflora. The same applies to all antimicrobial growth promoters, including antibiotics. The other important factor is the viability of the probiotic preparation; does it really contain the numbers of viable organisms that it claims to have? The viability of the preparation is not always checked before it is used for experimental trials. The data for field trials are therefore very difficult to evaluate but the fact that probiotics do on some occasions give positive results confirms their potential as growth promoters.

7.2 EFFECTS ON INTESTINAL INFECTIONS

The prolongation of the faecal excretion of salmonella in chickens fed antibiotics indicated the presence in the gut of a protective flora (Smith & Tucker 1975). This was confirmed by the finding that faecal organisms from adult chickens, when fed to newly hatched chicks, prevented colonization of the gut by *Salm. infantis* (Nurmi & Rantala 1973). Several groups throughout the world have subsequently confirmed the protective effect of the chick gut flora against salmonellas (Lloyd *et al.* 1977; Snoeyenbos *et al.* 1978; Huttner *et al.* 1981; Pivnick *et al.* 1981; Goren *et al.* 1984). Snoeyenbos and his group have demonstrated that the indigenous gut flora is also active against *E. coli* (Weinack *et al.* 1981), *Campylobacter fetus* subsp. *jejuni* (Soerjadi *et al.* 1982; Soerjadi Liem *et al.* 1984a), *Clostridium perfringens*, *Cl. botulinum* (Snoeyenbos *et al.* 1983) and *Yersinia enterocolitica* (Soerjadi Liem *et al.* 1984b). The specific bacteria responsible for this effect are still not known, although Mead and his group have been able to induce partial protection with a collection of 48 different bacteria including lactobacilli, streptococci, coliforms and strictly anaerobic bacteria (Impey *et al.* 1982).

Administration of crude faecal suspensions as enemas has been effective in combatting pseudomembranous colitis (Bowden *et al.* 1981; Schwan *et al.* 1984) which is a *Cl. difficile* infection associated with oral antibiotic therapy. Although this type of treatment is effective it is not one which would commend itself to clinicians and research continues in an attempt to identify the specific bacteria responsible for the effect. Preliminary trials with a lactobacillus preparation have shown promising results (Gorbach *et al.*, 1987) and *Bifid. longum* has been successfully used to reduce the after effects of erythromycin therapy (Colombel *et al.* 1987).

Another approach which has shown promising results is that of attempting to prevent colonization by occupying the niche required by the pathogen. Barrow & Tucker (1986) used this approach and discovered three strains of Gram-negative facultative anaerobes that effectively prevented colonization of the gut by salmonellas. Subsequently they obtained protection against *Salm. typhimurium* by pretreatment of the chickens with an avirulent strain of the same species (Barrow *et al.* 1987). While these results do illustrate the soundness of the approach, it is unlikely that this kind of organism could be used as a probiotic; the fear of reversion to a virulent type would preclude any commercial exploitation of these findings.

Similar trials with human babies showed that *per os* treatment with a strain of *E. coli* suppressed the development of a resistant *E. coli* population which occurred in the control group (Duval-Iflah *et al.* 1982). A non-pathogenic strain of *Cl. difficile* was used to protect hamsters against infection by this organism (Borriello & Barclay 1985). It was suggested that this might be an example of protection afforded by competition for adhesion sites.

Cole & Fuller (1984) approached the design of a probiotic by selection of a strain of lactobacillus on the basis of in-vitro tests for epithelial cell adhesion and bacterial antagonism. The strain of *L. salivarius* which they chose was able to suppress the growth of *E. coli* in the gut of new-born rats. In pigs a strain of *L. lactis* which reduced the count of *E. coli* in the gut was found to be associated with the small intestine epithelial surface (Muralidhara *et al.* 1977).

These studies tested the effect of lactobacillus preparations on the indigenous, non-pathogenic *E. coli* flora. Although this type of study showed positive effects on the *E. coli* flora, the evidence for the effectiveness of lactobacillus probiotics as antidiarrhoeal agents is not good. Attempts to protect against travellers diarrhoea (Pozo-Olano *et al.* 1978) and infantile diarrhoea (Pearce & Hamilton 1974) have proved unsuccessful but *L. acidophilus* protected new-born pigs against diarrhoea (Kohler & Bohl 1964).

Better results have been obtained with other probiotic organisms. A preparation containing *Bifidobacterium thermophilum* and *Bifid. pseudolongum* protected piglets against diarrhoea (Kimura *et al.* 1983). Young rabbits, which usually develop diarrhoea when dosed with a human isolate of pathogenic *E. coli*, can be protected by colonization of the gut with *Ent. faecium* (Wadstrom 1984). *Enterococcus faecium* fed before experimental challenge protected pigs against *E. coli* diarrhoea (Underdahl *et al.* 1982). The growth medium used affected the protective potential of the *Ent. faecium*; organisms grown in milk were effective but those grown in trypticase soy broth were not (Ushe & Nagy 1985). Under experimental conditions it was possible to protect pigs against infection with *E. coli* by dosing them with the corresponding bacteriophage. There was a reduction in scouring and in mortality following the treatment but it was considered too specific to be of any general use (Smith & Huggins 1983).

7.3 ALLEVIATION OF LACTOSE INTOLERANCE

Many people throughout the world suffer from lactose intolerance due to a congenital deficiency of the enzyme β-galactosidase resulting in the inability to digest lactose. It is known that lactose-intolerant people can digest lactose in yoghurt better than the same amount of lactose in milk (Gallagher *et al.* 1974). This has been confirmed by hydrogen breath analysis (Gilliland & Kim 1981). It has been suggested that yoghurt exerts its effect by supplying additional enzyme and there is evidence to support this. Rats fed yoghurt have increased concentrations of β-galactosidase in the small intestine (Garvie *et al.* 1984). This enzyme is of bacterial origin and is not due to stimulation of the rat lactase.

Milk containing *L. acidophilus* gave breath hydrogen values which were significantly lower than those from subjects ingesting unsupplemented milk (Gilliland & Kim 1982).

7.4 RELIEF OF CONSTIPATION

Some of the first clinical trials carried out with lactobacilli were related to their effect on constipation. Rettger & Cheplin (1921) favourably influenced the bowel function of patients by feeding them supplements of *L. acidophilus*. More recently Alm *et al.* (1983) and Graf (1983) have also had encouraging results in the use of acidophilus milk for the treatment of constipation.

7.5 ANTITUMOUR ACTIVITIES

Since the first observation by Bogdanov *et al.* (1962) that *L. bulgaricus* produced substances which were active against tumour development there have been a number of reports in the same vein.

The anticarcinogenic properties of lactobacilli fall into three categories; (a) the inhibition of tumour cells (Reddy *et al.* 1973), (b) the suppression of bacteria which produce enzymes such as β-glucosidase, β-glucuronidase and azoreductase (see above) which are responsible for the release of carcinogens from inocuous complexes (Goldin & Gorbach 1977) and (c) the destruction of carcinogens such as nitrosamines (Rowland & Grasso 1975) and the suppression of nitroreductase which is involved in the synthesis of nitrosamines (Goldin & Gorbach 1984).

7.6 ANTICHOLESTEROLAEMIC EFFECTS

The effect of yoghurt and acidophilus milk on blood cholesterol levels is variable. Feeding yoghurt to humans produced lower blood cholesterol concentrations (Mann 1977) but skim milk will also achieve the same result (Nair & Mann 1977). However, other work has shown that fermented milks give a lower serum cholesterol concentration than does untreated milk (Grunewald 1982). The suggestion was that fermented milk contained bacterial metabolites which inhibit cholesterol synthesis by the body. However, some lactobacilli have a direct effect on cholesterol levels by assimilation and removal from the growth medium. Feeding trials showed that such organisms significantly reduced cholesterol levels in the serum of pigs fed cholesterol (Gilliland *et al.* 1985).

Table 2 Features of a good probiotic

1. Should be a strain which is capable of exerting a beneficial effect on the host animal, e.g. increased growth or resistance to disease
2. Should be non-pathogenic and non-toxic
3. Should be present as viable cells, preferably in large numbers, although we do not know the minimum effective dose
4. Should be capable of surviving and metabolizing in the gut environment, e.g. resistant to low pH and organic acids
5. Should be stable and capable of remaining viable for long periods under storage and field conditions

8. Characteristics of a good probiotic

Although positive results can be demonstrated experimentally with probiotics, the results obtained in field trials have been variable. One of the problems is the nature of the phenomenon itself. It is bound to be variable because it operates by reversing stress factors which may or may not be present. This is particularly likely in the case of growth stimulation when the organism responsible for the growth depression is not always present in the gut. This sort of variation occurs with antibiotics and other chemical growth promoters. The practical consequence is that probiotics may work on one farm but not on another and on one occasion but not the next.

The other problem which has occurred with some of the commercial preparations is poor quality control. Clements *et al.* (1983) found that two batches of the same product gave different results when used to treat experimentally induced *E. coli* diarrhoea in human adults. Some preparations claiming to have viable cells present in large numbers have only very low numbers and others which claim to have one species of micro-organisms have a totally different species (Fowler 1969; Gilliland 1981). The features of a good probiotic are shown in Table 2. It should contain viable cells of the species specified on the label. Other features of an effective probiotic are that, of course, it should be non-pathogenic and not have adverse effects of any sort. It should do quite the opposite and have a beneficial effect in the form of growth promotion or increased resistance to disease. In order to produce the desirable effect it must be able to survive and metabolize in the intestine; it must therefore be resistant to low pH and all the other antibacterial influences present in the gut. The delivery of large numbers into the lower gut may be achieved either by feeding large numbers of viable cells continuously (e.g. as with yoghurt where the bacteria are non-intestinal and do not grow in the gut) or by restricted dosing with an intestinal strain which will colonize the gut and become self-replicating. It should also be remembered that the characteristics of the strain must survive growth on a large scale and be prepared in such a way as to retain its viability under storage and field conditions.

Such a probiotic with all these features has considerable advantages over antibacterial supplements currently in use. They do not induce resistance to antibiotics which will compromise therapy. They are not toxic and therefore will not produce undesirable side-effects when being fed and, in the case of food animals, will not produce toxic residues in the carcass. They may stimulate immunity, whereas the immune status remains unaffected by antibiotics. They may be cheaper; at present we do not know what the minimum effective dose is but if it can be established that the probiotic effects are obtained after minimal dosing then the cost will fall below that which it is at the moment when probiotics tend to consist of preparations containing large number of bacteria which are fed continuously.

However, the available evidence suggests that even with careful strain selection based on epithelial adhesion, growth rate and in-vitro bacterial antagonism, the effects produced after cessation of treatment are only of a limited duration. Using such a strain Cole & Fuller (1984) found that the effect on *E. coli* counts in neonatal rats was declining by seven days after treatment stopped. Similarly, in experiments with human patients fed a human strain of *L. acidophilus* the effect on enzyme activity had almost disappeared by 30 days after the treatment ended (Goldin & Gorbach 1984). In both cases, although there was some post-treatment effect it was transient. The lack of permanent

establishment of adhering strains in the pig gut was demonstrated by the work of Jonsson (1986) and Tannock (1989).

Although the production of a probiotic which would permanently colonize the gut and thus would require only a limited administration would be ideal, it may be difficult to achieve practically. Certainly in the adult where the intestinal lactobacillus niche is already occupied by naturally acquired strains the permanent establishment of a probiotic strain would require their displacement and would be difficult to induce. Even in the neonate where the flora is in a more unstable condition it would be necessary to administer the probiotic very soon after birth if it is to compete with the acquired flora.

It would seem, therefore, that the best method of administration is continuous feeding. This would ensure that the probiotic was present in the gut in large numbers and able to metabolize and produce its probiotic effect. However, even with continuous administration it is important to select strains with the maximum ability to survive in the intestine, and attention to colonization factors such as epithelial adhesion and growth rate is still recommended.

9. Future developments

Probiotics are in the early stages of use. Future developments will attempt to discover more effective strains. The selection of suitable strains can be done in three ways. It can be attempted by field testing a variety of naturally occurring strains; this is time consuming and expensive and not a practical solution. The number of strains for field testing may be limited by laboratory tests. Relevant information relates to antagonism for other bacteria, growth rate in the intestine (or diet) and ability to attach to gut epithelial cells. When using the last feature workers should be aware of the limitations of the information collected. The attachment to epithelial cells is very host specific which means in practical terms that a strain which is suitable for development as a pig probiotic may not be active in the chick and other animals. The degree of attachment is variable between strains of the same species so that although one strain of *L. acidophilus* is an effective probiotic, other strains of the same species may be totally unsuitable.

What is needed at the moment is more information on the way that probiotic supplements act. When we have this sort of information it may be possible to improve the strain by genetic manipulation. In this way it would be possible to bring together the ability to survive in the gut with the ability to produce the metabolites which are responsible for the probiotic effect. Recent work by McCarthy *et al.* (1988) suggests that the techniques are available. They showed that *L. acidophilus* isolated from pigs could be genetically transformed to enable them to colonize the mouse gastric epithelium. Although the technology is ready we still need to know what is to be manipulated by it. The current potential of this sort of approach has been reviewed by Tannock (1988b).

10. Summary

There is good evidence that the complex microbial flora present in the gastrointestinal tract of all warm-blooded animals is effective in providing resistance to disease. However, the composition of this protective flora can be altered by dietary and environmental influences, making the host animal susceptible to disease and/or reducing its efficiency of food utilization. What we are doing with the probiotic treatments is re-establishing the natural condition which exists in the wild animal but which has been disrupted by modern trends in conditions used for rearing young animals, including human babies, and in modern approaches to nutrition and disease therapy. These are all areas where the gut flora can be altered for the worse and where, by the administration of probiotics, the natural balance of the gut microflora can be restored and the animal returned to its normal nutrition, growth and health status.

11. References

ALM, L., HUMBLE, D., RYD-KJELLEN, E. & SETTERBERG, G. 1983 The effect of acidophilus milk in the treatment of constipation in hospitalised geriatric patients. *Symposia of Swedish Nutrition Foundation* **XV**, 131–138.

BAIRD, D.M. 1977 Probiotics help boost feed efficiency. *Feedstuffs* **49**, 11–12.

BARROW, P.A., BROOKER, B.E., FULLER, R. & NEWPORT, M.J. 1980 The attachment of bacteria to the gastric epithelium of the pig and its importance

in the microecology of the intestine. *Journal of Applied Bacteriology* **48**, 147–154.

BARROW, P.A. & TUCKER, J.F. 1986 Inhibition of colonization of the chicken caecum with *Salmonella typhimurium* by pre-treatment with strains of *Escherichia coli*. *Journal of Hygiene*, **96**, 161–169.

BARROW, P.A., TUCKER, J.F. & SIMPSON, J.M. 1987 Inhibition of colonization of the chicken alimentary tract with *Salmonella typhimurium* by Gram-negative facultatively anaerobic bacteria. *Epidemiology and Infection* **98**, 311–322.

BEALMEAR, P.M., HOLTERMANN, O.A. & MIRAND, E.A. 1984 Influence of the microflora on the immune response, I: General characteristics of the germ-free animal. In *The Germ-free Animal in Biomedical Research* ed. Coates, M.E. & Gustafsson, B.E., pp. 335–346. London: Laboratory Animals Ltd.

BERG, R.D. 1983 Translocation of indigenous bacteria from the intestinal tract. In *Human Intestinal Microflora in Health and Disease* ed. Hentges, D.J., pp. 333–352. London: Academic Press.

BHATTACHARYA, P.R. & MAJUNDER, M.K. 1983 Survival of orally administered isolated intestinal *Lactobacillus acidophilus* in different parts of gastrointestinal tract of mice. *Journal of Bioscience* **5**, 97–105.

BLOKSMA, N., ETTEKOVEN, H., HOTHUIS, F.M., VAN NOORLE-JANSEN, L., DE REUVER, M.J., KREEFLENBERG, J.G. & WILLERS, J.M. 1981 Effects of lactobacilli on parameters of non-specific resistance of mice. *Medical Microbiology & Immunology* **170**, 45–53.

BOGDONOV, I.G., POPKHIRSTOV, P. & MARINOV, L. 1962 Anticancer effect of antibioticum bulgaricum on sarcoma 180 and on the select form of Ehrlich carcinoma. Abstract VIII *International Cancer Congress* p. 364.

BOHNHOFF, M., DRAKE, B.L. & MILLER, C.P. 1954 Effect of streptomycin on susceptibility of the intestinal tract to experimental salmonella infection. *Proceedings of the Society for Experimental Biology and Medicine* **86**, 132–137.

BORRIELLO, S.P. & BARCLAY, F.E. 1985 Protection of hamsters against *Clostridium difficile* ileocaecitis by prior colonisation with non-pathogenic strains. *Journal of Medical Microbiology* **19**, 339–350.

BOWDEN, T.A., MANSBERGER, A.R. JR. & LYKINS, L.E. 1981 Pseudomembraneous Enterocolitis: Mechanism of restoring floral homeostasis. *American Surgery* **47**, 178–183.

CLEMENTS, M.L., LEVINE, M.M., RISTAINO, P.A., DAYA, V.E. & HUGHES, T.P. 1983 Exogenous lactobacilli fed to man—their fate and ability to prevent diarrhoeal disease. *Progress in Food and Nutrition Science* **7**, 29–37.

COLE, C.B. & FULLER, R. 1984 A note on the effect of host specific fermented milk on the coliform population of the neonatal rat gut. *Journal of Applied Bacteriology* **56**, 495–498.

COLLINS, F.M. & CARTER, P.B. 1978 Growth of Salmonellae in orally infected germfree mice. *Infection & Immunity* **21**, 41–47.

COLOMBEL, J.F., CORTOT, A., NEUT, C. & ROMOND, C. 1987 Yoghurt with *Bifidobacterium longum* reduces erythromycin-induced gastrointestinal effects. *Lancet* **ii**, 43.

CONWAY, P.L., GORBACH, S.L. & GOLDIN, B.R. 1987 Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *Journal of Dairy Science* **70**, 1–12.

DILWORTH, B.C. & DAY, E.J. 1978 Lactobacillus cultures in brooder diets. *Poultry Science* **57**, 1101.

DUBOS, R.J. 1963 Staphylococci and infection immunity. *American Journal of Diseases of Children* **105**, 643–645.

DUCLUZEAU, R., BELLIER, M. & RAIBAUD, P. 1970 Transit digestif de divers inoculums bacteriens introduits "Per os" chez des souris axeniques et "holoxeniques" (conventionelles): Effet antagoniste de la microflore du tractus gastro-intestinal. *Zentralblatt fur Bakterologie, Parasitenkunde, Infectionskrankheiten und Hygiene* Abt. I. Orig. **213**, S:533–548.

DUVAL-IFLAH, Y., OURIET, M-F., MOREAU, C., DANIEL, N., GABILAN, J-C. & RAIBAUD, P. 1982 Implantation of a strain of *Escherichia coli* in the digestive tract of human new-borns: barrier effect against antibioresistant *E. coli*. *Annals de Microbiologie* (*Institut Pasteur*) **133A**, 393–408.

FOWLER, G.G. 1969 *Lactobacillus acidophilus* or *Lactobacillus casei*. *Jahrgang* **24**, 211–213.

FRETER, R. 1955 The fatal enteric cholera infection in the guinea pig achieved by inhibition of normal enteric flora. *Journal of Infectious Diseases* **97**, 57–65.

FRETER, R. 1956 Experimental enteric shigella and vibrio infection in mice and guinea pigs. *Journal of Experimental Medicine* **104**, 411–418.

FRETER, R. 1962 *In vivo* and *in vitro* antagonism of intestinal bacteria against *Shigella flexneri* II The inhibitory mechanism. *Journal of Infectious Diseases* **110**, 38–46.

FRETER, R. & ABRAMS, G.D. 1972 Function of various intestinal bacteria in converting germfree mice to the normal state. *Infection and Immunity* **6**, 119–126.

FRIEND, B.A. & SHAHANI, K.M. 1984 Antitumor properties of lactobacilli and dairy products fermented by lactobacilli. *Journal of Food Protection* **47**, 717–723.

FULLER, R. 1973 Ecological studies on the lactobacillus flora associated with the crop epithelium of the fowl. *Journal of Applied Bacteriology* **36**, 131–139.

FULLER, R. 1975 Nature of the determinant responsible for the adhesion of lactobacilli to chicken crop epithelial cells. *Journal of General Microbiology* **87**, 245–250.

FULLER, R. 1978 Epithelial attachment and other factors controlling the colonization of the intestine of the gnotobiotic chicken by lactobacilli. *Journal of Applied Bacteriology* **46**, 335–342.

FULLER, R. 1982 Development and dynamics of the aerobic gut flora in gnotobiotic and conventional animals. *Advances in Veterinary Medicine* **33**, 7–15.

FULLER, R. & TURVEY, A. 1971 Bacteria associated with the intestinal wall of the fowl (*Gallus domesticus*). *Journal of Applied Bacteriology* **34**, 617–622.

FULLER, R., COLE, C.B. & COATES, M.E. 1984 The role of *Streptococcus faecium* in antibiotic relieved growth depression of chickens. In *Antibiotics in Agriculture: Benefits and Malefits* ed. Woodbine, M. pp. 395–403. London: Butterworths.

FULLER, R., NEWMAN, H.N. & SNOEYENBOS, G.H. 1986 Microbial competition in the mouth and gastrointestinal tract. In *Natural Antimicrobial Systems* ed. Gould, G.W., Rhodes-Roberts, M.E., Charnley, A.K., Cooper, R.M. & Board, R.G. pp. 11–28. Bath: Bath University Press.

GALLAGHER, C.R., MOLESON, A.L. & CALDWELL, J.H. 1974 Lactose intolerance and fermented dairy products. *Journal of American Diet Association* **65**, 418–419.

GARVIE, E.I., COLE, C.B., FULLER, R. & HEWITT, D. 1984 The effect of yoghurt on some components of the gut microflora and the metabolism of lactose in the rat. *Journal of Applied Bacteriology* **56**, 237–245.

GILLILAND, S.E. 1981 Enumeration and identification of lactobacilli in feed supplements marketed as sources of *Lactobacillus acidophilus*. *Oklahoma Agricultural Experimental Station Miscellaneous Publication* **108**, 61–63.

GILLILAND, S.E. & KIM, H.S. 1981 Influence of consuming milk containing cells of *Lactobacillus acidophilus* on lactose malabsorption in humans. *Abstracts of Annual Meeting of American Society of Microbiology* P27, p. 200.

GILLILAND, S.E. & KIM, H.S. 1982 Reduction of lactose malabsorption in humans by consumption of milk containing different numbers of *Lactobacillus acidophilus*. *Journal of Dairy Science* (Suppl. 1) 220.

GILLILAND, S.E., NELSON, C.R. & MAXWELL, C. 1985 Assimilation of cholesterol by *Lactobacillus acidophilus*. *Applied and Environmental Microbiology* **49**, 377–381.

GOLDIN, B.R. & GORBACH, S.L. 1977 Alterations in fecal microflora enzymes related to diet, age, lactobacillus supplements and dimethylhydrazine. *Cancer* **40**, 2421–2426.

GOLDIN, B.R. & GORBACH, S.L. 1984 The effect of milk and lactobacillus feeding on human intestinal bacterial enzyme activity. *American Journal of Clinical Nutrition* **39**, 756–761.

GORBACH, S.L., CHANG, T-W. & GOLDIN, B. 1987 Successful treatment of relapsing *Clostridium difficile* colitis with lactobacillus GG. *Lancet* **ii**, 1519.

GORBACH, S.L., BARZA, M., GIULIANO, M. & JACOBUS, N.V. 1988 Colonisation resistance of the human intestinal microflora: testing the hypothesis in normal volunteers. *European Journal of Clinical Microbiology and Infectious Diseases* **7**, 98–102.

GOREN, E., DE JONG, W.A., DOORNENBAL, P., KOOPMAN, J.P. & KENNIS, H.M. 1984 Protection of chicks against *Salmonella* infection induced by spray application of intestinal microflora in the hatchery. *Veterinary Quarterly* **6**, 73–79.

GRAF, W. 1983 Studies on the therapeutic properties of acidophilus milk. *Symposia of Swedish Nutrition Foundation* **XV**, 119–121.

GRUNEWALD, K.K. 1982 Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. *Journal of Food Science* **47**, 2078–2079.

HAN, I.K., LEE, S.C., LEE, J.H., LEE, K.K. & LEE, J.C. 1984a Studies on the growth promoting effects of probiotics I. The effects of *Lactobacillus sporogenes* in the growing performance and the change in microbial flora of the faeces and intestinal contents of the broiler chicks. *Korean Journal of Animal Science* **26**, 150–157.

HAN, I.K., LEE, S.C., LEE, J.H., KIM, J.D., JUNG, P.K. & LEE, J.C. 1984b Studies on the growth promoting effects of probiotics II. The effects of *Clostridium butyricum ID* on the performance and the changes in the microbial flora of the faeces and intestinal contents of the broiler chicks. *Korean Journal of Animal Science* **26**, 158–165.

HAN, I.K., KIM, J.D., LEE, J.H., LEE, S.C., KIM, T.H. & KWAG, J.H. 1984c Studies on the growth promoting effects of probiotics III. The effects of *Clostridium butyricum ID* on the performance and the changes in the microbial flora of the faeces of growing pigs. *Korean Journal of Animal Science* **26**, 166–171.

HENTGES, D.J. 1983 Role of the intestinal microflora in host defense against infection. In *Human Intestinal Microflora in Health and Disease* ed. Hentges, D.J. Ch. 14, pp. 311–331. New York: Academic Press.

HOUGHTON, S.B., FULLER, R. & COATES, M.E. 1981 Correlation of growth depression of chicks with the presence of *Streptococcus faecium* in the gut. *Journal of Applied Bacteriology* **51**, 113–129.

HUPPERT, M., CAZIN, J. & SMITH, H. 1955 Pathogenesis of *Candida albicans* in the intestinal tract of mice. *Journal of Bacteriology* **70**, 440–447.

HUTTNER, B., LANDGRAF, H. & VIELITZ, E. 1981 Control of Salmonella infections in broiler breeder flocks by administration of SPF chicken intestinal flora to day-old chicks. *Deutsch Tierarztl Wochenschrift* **88**, 527–532.

IMPEY, C.S., MEAD, G.C. & GEORGE, S.M. 1982 Competitive exclusion of salmonellas from the chick caecum using a defined mixture of bacterial isolates from the caecal microflora of an adult bird. *Journal of Hygiene* **89**, 479–490.

JONSSON, E. 1986 Persistence of *Lactobacillus* strain in the gut of suckling piglets and its influence on performance and health. *Swedish Journal of Agricultural Research* **16**, 43–47.

KATO, I., KOBAYASHI, S., YOKOKURA, T. & MUTAI, M. 1981 Antitumour activity of *Lactobacillus casei* in mice. *Gann* **72**, 517–523.

KATO, I., YOKOKURA, T. & MUTAI, M. 1983 Macrophage activation by *Lactobacillus casei* in mice. *Microbiology & Immunology* **27**, 611–618.

KIMURA, N., YASHIKANE, M., KOBAYASHI, A. & MITSUOKA, T. 1983 An application of dried bifidobacteria preparation to scouring animals. *Bifidobacteria Microflora* **2**, 41–55.

KOHLER, E.M. & BOHL, E.M. 1964 Prophylaxis of diarrhoea in newborn pigs. *Journal of the American Veterinary Medical Association* **144**, 1794–1797.

LENCNER, A.A., LENCNER, Ch.P. & MIKELSAAR, M.E. 1984 The quantitative composition of the gut lactoflora before and after cosmic flights of different duration. *Die Nahrung* **28**, 607–613.

LILLY, D.M. & STILLWELL, R.H. 1965 Probiotics: Growth promoting factors produced by microorganisms. *Science* **147**, 747–748.

LIZKO, N.N., SILOV, V.M. & SYRYCH, G.D. 1984 Particularities in the formation of an intestinal dysbacteriosis in man under extreme conditions. *Die Nahrung* **28**, 599–605.

LLOYD, A.B., CUMMING, R.B. & KENT, R.D. 1977 Prevention of *Salmonella typhimurium* infection in poultry by pretreatment of chickens and poults with intestinal extracts. *Australian Veterinary Journal* **53**, 82–87.

MCCARTHY, D.M., LIN, J.H.C., RINCKEL, L.A. & SAVAGE, D.C. 1988 Genetic transformation in *Lactobacillus* sp. Strain 100-33 of the capacity to colonize the nonsecreting gastric epithelium in mice. *Applied and Environmental Microbiology* **54**, 416–422.

MANN, G.V. 1977 A factor of yoghurt which lowers cholesterolaemia in man. *Atherosclerosis* **26**, 335–340.

MAIER, B.R. & HENTGES, D.J. 1972 Experimental *Shigella* infections in laboratory animals I. Antagonism by normal flora components in gnotobiotic mice. *Infection and Immunity* **6**, 168–173.

MILES, R.D., ARAFA, A.S., HARMS, R.H., CARLSON, C.W., REID, B.L. & CRAWFORD, J.S. 1981 Effects of a living non-freeze dried *Lactobacillus acidophilus* culture on performance, egg quality and gut microflora in commercial layers. *Poultry Science* **60**, 993–1004.

MOORE, W.E.C. & HOLDMAN, L.V. 1974 Human fecal flora: The normal flora of 20 Japanese Hawaiians. *Applied Microbiology* **27**, 961–979.

MORDENTI, A. 1986 Probiotics and new aspects of growth promoters in pig production. *Information Zootechnology* **32**, 69.

MURALIDHARA, K., SHEGGEBY, G.G. & ELLIKER, P.R., ENGLAND, D.C. & SANDINE, W.E. 1977 Effect of feeding lactobacilli on the coliform and lactobacillus flora of intestinal tissue and faeces from pigs. *Journal of Food Protection* **40**, 288–295.

NAIR, C.R. & MANN, G.V. 1977 A factor in milk which influences cholesterolaemia in rats. *Atherosclerosis* **26**, 363–367.

NURMI, I.E. & RANTALA, M. 1973 New aspects of *Salmonella* infection in broiler production. *Nature* **241**, 210–211.

PARKER, R.B. 1974 Probiotics, the other half of the antibiotics story. *Animal Nutrition and Health* **29**, 4–8.

PEARCE, J.L. & HAMILTON, J.R. 1974 Controlled trial of orally administered lactobacilli in acute infantile diarrhoea. *Journal of Pediatrics* **84**, 261–262.

PERDIGON, G., DE MACIAS, M.E.N., ALVAREZ, S., OLIVER, G. & DE RUIZ HOLGADO, A.A.P. 1986 Effect of perorally administered lactobacilli on macrophage activation in mice. *Infection and Immunity* **53**, 404–410.

PIVNICK, H., BLANCHFIELD, B. & D'ANST, J-Y. 1981 Prevention of *Salmonella* infection in chicks by treatment with fecal cultures from mature chickens (Nurmi cultures). *Journal of Food Protection* **44**, 909–916.

POLLMAN, D.S. 1986 Additives, flavors, enzymes and probiotics in animal feeds. *Proceedings of 22nd Annual Nutrition Conference*. University of Guelph.

POLLMANN, D.S., DANIELSON, D.M. & PEO, E.R. 1980 Effects of microbial feed additives on performance of starter and growing-finishing pigs. *Journal of Animal Science* **51**, 577–581.

POZO-OLANO, J.D., WANAN, J.H., GOMEZ, R.G. & CAVAZOS, M.G. 1978 Effect of a lactobacilli preparation on travellers diarrhoea. *Gastroenterology*, **74**, 829–830.

RATCLIFFE, B., COLE, C.B., FULLER, R. & NEWPORT, M.J. 1986 The effect of yoghurt and milk fermented with a porcine intestinal strain of *Lactobacillus reuteri* on the performance and gastrointestinal flora of pigs weaned at two days of age. *Food Microbiology* **3**, 203–211.

REDDY, G.V., FRIEND, B.A., SHAHANI, K.M. & FARMER, R.E. 1983 Antitumor activity of yoghurt components. *Journal of Food Protection* **46**, 8–11.

REDDY, G.V., SHAHANI, K.M. & BANERJEE, M.R. 1973 Inhibitory effect of the yoghurt on Ehrlich ascites tumor cell proliferation. *Journal of the National Cancer Institute* **50**, 815–817.

RETTGER, L.F. & CHEPLIN, H.A. 1921 *A Treatise on the Transformation of the Intestinal Flora with Special Reference to the Implantation of* Bacillus acidophilus. New Haven, Connecticut: Yale University Press.

ROACH, S. & TANNOCK, G.W. 1980 Indigenous bacteria that influence the number of *Salmonella typhimurium* in the spleen of intravenously challenged mice. *Canadian Journal of Microbiology* **26**, 408–411.

ROWLAND, I.R. & GRASSO, P. 1975 Degradation of N-nitrosamine by intestinal bacteria. *Applied Microbiology* **29**, 7–12.

SAITO, H., TOMIOKA, H. & SATO, K. 1981 Enhanced resistance of *Lactobacillus* against *Listeria* infection in mice. *Medicine and Biology* **102**, 273–277.

SCHWAN, A., SJOLIN, S., TROTTESTAM, U. & ARONSSON, B. 1984. Relapsing *Clostridium difficile* enterocolitis cured by rectal infusion of normal faeces. *Scandinavian Journal of Infectious Diseases* **16**, 211–215.

SEELIG, M.S. 1966 Mechanisms by which antibiotics increase the incidence and severity of candidiasis and alter the immunological defenses. *Bacteriological Reviews* **30**, 444–459.

SMITH, H.W. 1965 The development of the flora of the alimentary tract in young animals. *Journal of Pathological Bacteriology* **90**, 495.

SMITH, H.W. & HUGGINS, M.B. 1983 Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. *Journal of General Microbiology* **129**, 2659–2675.

SMITH, H.W. & TUCKER, J.F. 1975 The effect of feeding diets containing permitted antibiotics on the faecal excretion of *Salmonella typhimurium* by experimentally infected chicks. *Journal of Hygiene, Cambridge* **75**, 293–301.

SNOEYENBOS, G.H., WEINACK, O.M. & SMYSER, C.F. 1978 Protecting chicks and poults from salmonellae by oral administration of normal gut microflora. *Avian Diseases* **22**, 273–287.

SNOEYENBOS, G.H., WEINACK, O.M. & SOERJADI, A. 1983 Competitive exclusion of some pathogens other than salmonella by native intestinal microflora of chickens. *Proceedings of 22nd World Veteri-*

nary Congress p. 191. Perth, Australia.

SOERJADI, A.S., SNOEYENBOS, G.H. & WEINACK, O.M. 1982 Intestinal colonization and competitive exclusion of *Campylobacter fetus* subsp. *jejuni* in young chicks. *Avian Diseases* **26**, 520–524.

SOERJADI LIEM, A.S., SNOEYENBOS, G.H. & WEINACK, O.M. 1984a Comparative studies on competitive exclusion of three isolates of *Campylobacter fetus* subsp. *jejuni* in chickens by native gut microflora. *Avian Diseases* **28**, 139–146.

SOERJADI LIEM, A.S., SNOEYENBOS, G.H. & WEINACK, O.M. 1984b Establishment and competitive exclusion of *Yersinia enterocolitica* in the gut of monoxenic and holoxenic chicks. *Avian Diseases* **29**, 256–260.

STAVRIC, S., GLEESON, T.M., BLANCHFIELD, B. & PIVNICK, P. 1987 Role of adhering microflora in competitive exclusion of *Salmonella* from young chicks. *Journal of Food Protection* **50**, 928–932.

TANNOCK, G.W. 1983 The effect of dietary and environmental stress on the gastrointestinal microbiota. In *Human Intestinal Microflora in Health and Disease* ed. D.J. Hentges, pp. 517–539. New York: Academic Press.

TANNOCK, G.W. 1988a The normal microflora: new concepts in health promotion. *Microbiological Sciences* **5**, 4–8.

TANNOCK, G.W. 1988b Molecular genetics: a new tool for investigating the microbial ecology of the gastrointestinal tract. *Microbial Ecology* **15**, 239–256.

TANNOCK, G.W. 1989 Colonization of the porcine gastrointestinal tract by lactobacilli. *Applied and Environmental Microbiology* **55**, 279–283.

TEN BRINK, B., MINEKUS, M., BOL, J. & HUIS IN T'VELD, J.H.J. 1987 Production of antibacterial compounds by lactobacilli. *FEMS Microbiology Reviews* **46**, 64.

TOMODA, T., NAKANO, Y. & KAGEYAMA, T. 1983 Variation of intestinal *Candida* of patients with leukemia and the effect of *Lactobacillus* administration. *Japanese Journal of Medical Mycology* **24**, 356–358.

UNDERDAHL, N.R., TORRES-MEDINA, A. & DOSTER, A.R. 1982 Effect of *Streptococcus faecium* C63 in control of *Escherichia coli*-induced diarrhoea in gnotobiotic pigs. *American Journal of Veterinary Research* **43**, 2227–2232.

USHE, T.C. & NAGY, B. 1985 Inhibition of small intestinal colonization of enterotoxigenic *Escherichia coli* by *Streptococcus faecium* M74 in pigs. *Zentralblatt fur Bakteriologie Parasitenkunde Infectionskrankheuten und Hygiene* Abt I, Orig. B **181**, 374–382.

VAN DER WAAIJ, D., BERGHUIS-DE VRIES, J.M., LEKKERKERK-VAN DER WEES, J.E.C. 1971 Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. *Journal of Hygiene* **69**, 405–411.

WADE, S., CORTHIER, G., MOREAU, L. & BESNIER, M.O. 1984 L'ingestion de yaout vivant modifie-t-elle la response immunitaire? *IDF Bulletin* no. 179 p.I.

WADSTROM, T. 1984 *Streptococcus faecium* M74 in control of diarrhoea induced by a human enterotoxigenic *Escherichia coli* strain in an infant rabbit model. *Zentralblatt für Bakteriologie Mikrobiologie und Hygiene* **A257**, 357–363.

WEINACK, O.M., SNOEYENBOS, G.H., SMYSER, C.F. & SOERJADI, A.S. 1981 Competitive exclusion of intestinal colonization of *Escherichia coli* in chicks. *Avian Diseases* **25**, 696–705.

WILSON, K.H. & PERINI, F. 1988 Role of competition for nutrients in suppression of *Clostridium difficile* by the colonic microflora. *Infection and Immunity* **56**, 2610–2614.