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Potential of Probiotic Organisms to Prevent Diarrhoea and Promote Digestion in Farm Animals—A Review

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ABSTRACT

This review examines the scientific basis for claims that commercial probiotics prevent diarrhoea and increase digestive performance in young farm animals. Suppression of pathogenic coliforms in the stomach and intestine by competitive growth and bacteriocidal secretions of lactic acid bacteria justifies the use of lactobacilli in probiotics. However, there is as yet insufficient evidence to support the notion of a beneficial digestive role for this gut microflora.

Key words: Probiotics, diarrhoea, digestion.

INTRODUCTION

In common with other species, the alimentary tract of the porcine foetus is sterile until birth (Fuller 1962; Gordon and Pesti 1971). Thereafter contact with the sow and the environment leads to successional colonisation by a variety of microbes (Smith 1965; Contrepois and Gouet 1973). This establishment of bacterial activity is regarded as complementary to the digestive functions of the host by extending the range of digestive enzymes and, under normal conditions, providing a barrier against invading pathogens. For instance, dosing newly hatched chicks with faeces from adult birds prevented salmonellas from colonising the caecae (Nurmi and Rantala 1973). In contrast, germ-free mice or animals dosed with large amounts of antibiotics show enhanced susceptibility to salmonella infection compared with conventional rodents (Bohnhoff *et al* 1954; Collins and Carter 1978).

At times of stress, such as weaning, the 'balance' of intestinal microflora may become disturbed and disorders in digestive function are likely to occur. When mice

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were deprived of feed, water and bedding for 2 days, large numbers of salmonellas and coliforms were found to proliferate in their intestines, whilst counts of lactobacilli were depressed (Tannock and Savage 1974). Similar changes were found in the anterior intestinal tract of piglets after they were abruptly weaned at 2 days, rather than being allowed to suckle (Barrow *et al* 1977). They also suffered from a high incidence of diarrhoea (scouring). Miller *et al* (1985) have also reported gut colonisation of entero-toxic *Escherichia coli* and severe diarrhoea to occur when piglets were weaned at 3 weeks of age on to a soya-based diet.

Antibiotics and chemotherapeutic agents are widely used as feed additives to inhibit pathogenic bacteria. Unfortunately such treatment can also depress non-pathogenic bacteria. Adding large amounts of penicillin to the diet of chickens suppressed *Lactobacillus* counts in the crop and allowed *E coli* numbers to increase (Fuller 1973). There is also a risk that indiscriminate use of antibiotics will lead to drug-resistant strains of *E coli* which may transfer resistance to other pathogens.

Growing public disquiet over the use of antibiotics in feed additives has encouraged recent commercial interest in probiotics as an alternative therapy. A number of commercial preparations are available which aim to promote colonisation of desirable bacteria in the gut by application of 'live' microorganisms (not necessarily indigenous to the host) and/or mixtures of metabolites needed to support their growth.

The purpose of this paper is (1) to survey the potential of probiotic organisms as dietary adjuncts for preventing diarrhoea and improving growth, (2) to examine the feasibility of manipulating intestinal microflora by administration of indigenous bacteria, and (3) to consider reasons for inconsistency in responses from probiotics together with possible improvements in their design.

PROBIOTIC CONCEPT

The origin of the term 'probiotic' is attributed to Parker (1974) who defined them as 'organisms and substances which contribute to intestinal microbial balance'. In a recent review of probiotics, Fuller (1986) considered this definition to be too broad since, besides including cultures, cells and metabolites of microbes, it could encompass antibiotic preparations. However, the concept of microfloral manipulation was first appreciated by Metchnikoff (1907) who viewed the consumption of yoghurt by Bulgarian peasants as conferring a long span of life. Although evidence for a link between longevity and ingestion of fermented milk has not been proven, some workers have claimed that its therapeutic value is related to viable bacteria, in particular *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Goodenough and Kleyn 1976; Speck 1977).

PROTECTION AGAINST ENTERIC PATHOGENS

Since Metchnikoff's early work, studies in several species have shown the ability of lactobacilli to suppress coliform growth. Feeding viable cells of *Lactobacillus*

acidophilus to young dairy calves was shown to reduce the incidence of diarrhoea (Bechman *et al* 1977), and increase the numbers of lactobacilli and reduce coliform counts in faeces (Bruce *et al* 1979). These findings contrast with those of other workers who have not observed benefits from feeding either *L acidophilus* (Hatch *et al* 1973; Ellinger *et al* 1978) or milk cultured with *L acidophilus* or *L lactis* (Morrill *et al* 1977).

In a detailed microbiological study by Muralidhara *et al* (1977), piglets given an *L lactis* concentrate for up to 8 weeks after birth showed a progressive decline in coliform counts in faecal samples. But this was not accompanied by a notable change in numbers of lactobacilli. Even so, suppression of coliforms was maintained for a 30-day period following discontinuation of treatment with the lactobacilli. Scouring in these animals was negligible, but was evident in control pigs especially at weaning.

In addition to lactobacilli some attention has been given to streptococci. Underdahl *et al* (1982) observed only mild diarrhoea lasting 2–4 days in gnotobiotic pigs inoculated with *Streptococcus faecium* prior to artificial *E coli* infection. In the same study persistent diarrhoea occurred in pigs similarly infected with *E coli*, but without prophylactic treatment with the *Streptococcus* organism.

MODES OF ACTION

Several mechanisms have been investigated whereby lactic acid bacteria could inhibit colonisation of the intestine by coliforms. These include adhesion to the digestive tract wall to prevent colonisation of pathogens, neutralisation of toxins, bacteriocidal activity, prevention of amine synthesis and enhanced immune competence.

Competitive attachment

Surface action through attachment to the intestinal wall is necessary for enterotoxin-producing strains of *E coli* to induce diarrhoea (Jones and Rutter 1972). Thus the idea that lactobacilli compete with coliforms for sites of adherence on the intestinal surface is an attractive hypothesis; attachment is believed to support proliferation and reduce peristaltic removal of organisms. Evidence supporting competitive attachment between lactic acid bacteria and coliforms was reported by Muralidhara *et al* (1977) who found that homogenates of washed intestinal tissue collected from piglets dosed with *L lactis* had markedly higher numbers of attached lactobacilli and lower *E coli* counts than scouring or normal control pigs.

The ability of bacteria to adhere to squamous epithelial cells appears to depend on attraction between an acidic mucopolysaccharide forming the outer layer of the bacterial cell wall and a similar coating on epithelial cells (Fuller and Brooker 1974). Fibrils are commonly found on adhering bacteria and may reinforce attachment, although adhesion can occur *in vitro* in their absence. See Fuller and Brooker (1980) for a detailed review of attachment mechanisms.

Rapid growth of bacteria in digesta may compensate, to some extent, for their failure to adhere to epithelial cells. Species such as *S faecium* and *E coli* showed little

or no ability to adhere to gastric epithelial tissue, but they were found to exhibit a superior rate of multiplication in sow's milk when compared with tissue-adhering strains of lactobacilli (Fuller 1986).

Bacteriocidal activity

In the suckling pig, attachment of lactobacilli bacteria to the pars oesophagus is thought to inoculate ingested milk (Fuller and Brooker 1980). Production of lactic acid by these organisms reduces the pH of the stomach contents. Studies *in vitro* have shown that an acid condition of less than pH 4·5 prevents the growth of many bacteria, including coliforms, but still allows the growth of some strains of lactobacilli (Fuller 1977; Barrow *et al* 1977). Rearing piglets from 2 days of age on an artificial milk treated with lactic acid lowered the pH of gastric contents, reduced counts of haemolytic *E coli* in the stomach and lessened the numbers of days of scouring compared with piglets given untreated milk (White *et al* 1969). In a similar experiment lactic acid was found to depress counts of coliforms and lactobacilli throughout the alimentary tract of piglets (Ratcliffe *et al* 1986).

Hydrochloric acid production in the stomach is also believed to have bacteriocidal properties for certain microorganisms, in particular coliforms. However, according to Cranwell *et al* (1976) young piglets do not produce significant quantities of hydrochloric acid until they reach 3–4 weeks of age. Consequently their protection against growth of coliforms in the stomach may depend, in part, on acid conditions produced by lactic acid bacteria.

Lactobacilli are also known to produce hydrogen peroxide which has bacteriocidal actions *in vitro* (Reiter *et al* 1980) or can activate the lactoperoxidase-thiocyanate (LP) system (see Reiter 1978). A strain of *L lactis*, chosen for its ability to produce hydrogen peroxide, was reported to activate the LP system in the abomasum of calves (Reiter *et al* 1980). Evidence for the efficacy of the LP system was demonstrated by inoculating calves with *L lactis* and *E coli*. Appreciable survival of coliform bacteria only occurred if the equilibrium of the LP system was reversed by addition of a reducing agent. It is unknown if the LP system is present in the suckling pig.

Enterotoxin neutralisation

Investigations of *L bulgaricus* in pigs showed that the organism produces a metabolite thought to neutralise the effect of enterotoxin released from coliforms. Piglets fed an *L bulgaricus* culture rendered non-viable with lactic acid, and artificially infected with *E coli*, grew faster and suffered less diarrhoea compared with control animals (Mitchell and Kenworthy 1976). Although the neutralising substance has yet to be identified, further support for anti-enterotoxic activity has also been obtained from experiments with rats and calves (Stuart *et al* 1978; Schwab *et al* 1980). Cell-free extracts of *L casei* and *L acidophilus* have also been shown *in vitro* to inhibit the growth of *E coli* (Mitchell and Kenworthy 1976; Hosono and Tokita 1977).

Prevention of toxic amine synthesis

Coliforms and certain other bacteria in the pig gut have the ability to decarboxylate

amino acids and could yield amines having toxic properties (Hill *et al* 1970a) or perhaps cause subclinical pharmacological effects. Although in-vivo toxicity has not been demonstrated, increased production of amines in the intestinal tract of young pigs has been observed shortly after weaning and noted to coincide with the onset of diarrhoea (Hill *et al* 1970b).

Enhanced immunity

At weaning, immunity resulting from gut exposure to a variety of antigens, such as pathogenic bacteria and dietary protein, is important in the defence of young animals against enteric infection (Porter *et al* 1977; Newby *et al* 1984). Recent observations of raised activities of macrophages and lymphocytes in mice following oral inoculation or intraperitoneal injection with lactobacilli imply an immunopotentiating role for lactic acid bacteria in the gut (Perdigon *et al* 1986). Evidence of immunostimulation by microorganisms was also observed in piglets. Oral inoculation of germ-free animals with *L acidophilus* led to elevated levels of total serum protein, apparently globulin rather than albumin, and increased white blood cell counts (Pollmann *et al* 1980).

However, the extent to which lactobacilli act as adjuvants in the immune defence system of the host is uncertain. But lactobacilli could be important in the development of immune competence in young piglets, particularly during weaning when protection must be acquired against antigens likely to cause gut inflammatory reactions (Miller *et al* 1985).

DIGESTIVE ACTIVITY

Commercial information about probiotics often includes the claim that, besides protecting animals against enteric infection, feeding these bacterial preparations results in increased feed conversion efficiency and live weight gain. However, this literature does not indicate whether growth responses stem directly from improved digestive performance or indirectly consequent to the suppression of gut pathogens which might otherwise have adverse effects on gastrointestinal function or metabolism.

Carbohydrates

Undoubtedly bacteria are essential for digestion in animals which rely on forestomach fermentation. Less certain are the digestive contributions made by intestinal bacteria in the fore and hind gut of monogastrics. It is feasible that extracellular enzymes of gut microflora supplement endogenous secretions, particularly during early life when the digestive system is immature (Kidder and Manners 1978). Szabo (1979) found elevated activities of lactase and lipase in the large intestine of conventional piglets compared with germ-free animals. But the same study provided indirect evidence that gut microflora might also inactivate endogenous enzymes. For example, higher levels of peptidase and disaccharidase

activities were found in the small and large intestines of germ-free than in conventional piglets.

Nevertheless, significant opportunities for microbial degradation of dietary nutrients occur in the stomach where simple sugars are known to be fermented to lactic acid (Ratcliffe 1985), and in the large bowel where a variety of organisms, mainly anaerobic gram-negative species, are capable of fermenting complex carbohydrates and yielding volatile fatty acids. For a detailed review of carbohydrate fermentation in the pig see Longland *et al* (1989).

Lactose intolerance

Several intolerance syndromes are known to arise from a deficiency of intestinal surface di- and oligosaccharidases (see Lifshitz 1984). For instance, lactase deficiency is commonly found in man and gives rise to a complex of digestive malfunctions such as gastric distension, abdominal cramp and watery diarrhoea. The symptoms are thought to relate to the metabolism of excess lactose by bacteria. Fermentation products, notably short-chain volatile fatty acids, hydrogen and carbon dioxide, accumulate in the lower gut and colon where they cause hyperosmotic effects and increase intraluminal gas pressure (Gray 1984).

The symptoms experienced by lactase-deficient subjects after ingesting lactose do not occur when they consume yoghurt (Savaiano *et al* 1984). This apparent benefit has been attributed to bacterial β-galactosidase activity in the yoghurt (Speck 1983; Kolars *et al* 1984). Whether the beneficial effects of yoghurt are entirely due to the effects of microbial enzymes is uncertain since other factors besides improved lactose digestion appear to be involved in the alleviation of the disorder. Savaiano *et al* (1984) noted that test meals containing a mixture of pasteurised yoghurt and lactose were tolerated by lactose-sensitive subjects despite evidence of disaccharide malabsorption and the fact that little or no lactase was detected in the heated yoghurt. Further doubt about a possible digestive benefit of yoghurt consumption arose from a study reported by Cole *et al* (1984) in which chickens (which do not produce endogenous lactase) were used as a model of lactose intolerance. Lactose feeding did not cause a change in numbers of caecal bacteria known to use lactose. This treatment did reduce growth rate and induce gas formation, although curiously these effects were not altered by adding yoghurt to the diet.

It is also uncertain whether the beneficial effects of yoghurt are linked with a suppression of deleterious organisms. In further experiments with chickens, Cole *et al* (1984) observed that the addition of yoghurt, either heated or unheated, to a diet containing lactose markedly reduced β-glucuronidase activity of caecal digesta compared with that of caecal contents from young birds fed lactose alone. These treatments did not, however, have a significant effect on caecal counts of lactobacilli and coliforms. This contrasts with a study of young piglets in which yoghurt feeding was found to depress numbers of coliforms and increase the *Lactobacillus* count in contents of the stomach and small bowel compared with bacteria in gastric digesta from animals given feeds based on skim milk and butterfat (Ratcliffe *et al* 1986). The authors suggested that, since bacterial counts in the stomach were also reduced when the skim milk was acidified with lactic acid, the yoghurt may have acted by lowering the pH of gastric contents.

Fats

Gut microflora may also affect the availability of energy to the host by interactions with fat metabolism. It is noteworthy that suppression of gut bacteria by administration of antibiotics was shown to improve fat digestibility in growing pigs (Mason and Just 1976). The effect was linked to improved fat absorption in the hind gut, suggesting that fat-metabolising bacteria reduce the availability of dietary lipid to the host. Ratcliffe (1985) proposed that microbial hydrogenation could increase the amounts of stearic acid, which is less well absorbed than unsaturated fatty acids. Impaired lipid absorption is also thought to occur as a result of deconjugation of bile acids by gut flora (Eyssen and de Somer 1967). This action on bile acids was suggested as a reason for the growth-depressing effect of *S faecium* on germ-free chickens (Cole *et al* 1981). However, deconjugation of bile acids was also shown with strains of lactobacilli (*L acidophilus* and *L fermentum*), but these organisms did not inhibit the growth of chickens (Cole and Fuller 1984).

Proteins

Amino acids which are potentially available to the host may be lost as a result of microbial deamination. This process could be serious if it affected essential amino acids in the small intestine. Indirect estimates of the extent of deamination by gut microflora of pigs has been studied *in vitro* by Buraczewska and Buraczewski (1985). Incubation of a casein hydrolysate (as a source of amino acids) with digesta collected from the ileum or caecum decreased total amino acids by 8% and 24% respectively. Marked depressions were found for contents of aspartic and glutamic acids, serine, lysine, histidine and arginine in ileal digesta, whilst all of the added amino acids were deaminated to some extent during incubation with caecal digesta. In similar studies of digesta collected from the small intestine of piglets, Hill *et al* (1970a,b) observed increased deaminative activity to occur within 24 h of weaning. Apparent breakdown of amino acids was enhanced by adding lactobacilli to the diet. However, comparisons of net nitrogen absorption of different proteins by germ-free and conventional chickens suggest that intestinal microflora do not compete with the host for amino acid nitrogen (Salter *et al* 1974).

Bacteria possessing the enzyme urease are likely to degrade recycled urea which enters the gut lumen from the circulation system. But since urea is a metabolic end-product, its breakdown by bacteria is likely to be of little consequence to the host. However, release of ammonia in excess could be sufficiently toxic to affect nutrient absorption and metabolism in the liver (Visek 1978).

Of course, any digestive contribution by microflora to the nutritional requirements of the host must be balanced against losses resulting from dietary components becoming bound in microbial cells and voided in faeces. Understanding of the relative digestive activities of different gut organisms is needed before a rational selection can be made for their application as probiotics. It is not intended to review the digestive enzyme activities of gut microflora here, but rather to consider possible benefits to the nutrition of the host. Information on microbial enzymes is provided in an excellent paper by Ratcliffe (1985).

Anti-nutritional factors

Sources of non-milk protein (eg soya bean meal) used as ingredients of animal diets may contain antinutritional factors such as lectins, enzyme inhibitors and allergens. Some of these substances have been implicated in digestive disorders in calves and piglets (Sissons 1982; Miller *et al* 1985). Susceptibility to these harmful substances may stem from an inability of young animals to digest the biologically active structures (Sissons and Thurston 1984). Such defects might be overcome by digestive or detoxifying actions of microflora, but so far there is little evidence to support this idea. Incubation *in vitro* of soya bean meal with a probiotic based on lactic acid bacteria reduced the antigenic activity of one major storage protein, β-conglycinin, although this treatment had no effect on the immunological activity of another soya globulin, glycinin (Sissons J W unpublished observations). Moreover, microorganisms in the gut appear to have no effect on digestive disorders linked with protease inhibitors. Coates *et al* (1970) noted that chickens reared in germ-free or conventional environments showed similar responses of pancreatic enlargement and hypersecretion to a diet containing unheated soya bean meal.

INCONSISTENT EFFICACY OF PROBIOTICS

There is a wealth of commercial literature which claims that feeding probiotics to pigs will overcome disorders of stress and improve growth rates. Unfortunately scientific trials using probiotic organisms have often failed to demonstrate beneficial effects by altering the balance between lactic acid bacteria and coliforms, by controlling diarrhoea or by enhancing growth performance (Hill *et al* 1970a; Pollmann *et al* 1980; Jonsson and Olsson 1985). In a recent review of probiotic efficacy Fuller (1986) proposed several reasons why probiotic organisms might fail to achieve a beneficial response. These included non-adherence to gastric and gut epithelial tissue, inability to grow in the gut environment and a lack of specificity for the host.

Growth in diet and digesta

Few studies have examined the influence of dietary constituents on growth of probiotic organisms. According to Brockett and Tannock (1981), long-chain fatty acids, particularly unsaturated molecules, are recognised as having an inhibitory effect on bacterial growth. In studies of lactobacilli attachment to non-secretory epithelial tissue of the stomach of mice, increasing the ratio of palmitic to oleic acid led to a reduction in numbers of adhering organisms. In experiments with calves, milk fat was not found to affect numbers of lactobacilli in digesta collected from different regions throughout the gastrointestinal tract. However, addition of 32 mM propionic acid to a milk feed depressed lactobacilli counts by nearly 1000-fold. The reason for this suppressive effect by propionate is uncertain.

For microorganisms to survive and grow in the intestine they need to be tolerant of bile. A comparison of the ability of several strains of *L acidophilus* to grow in a broth with and without bile juice showed considerable variation among strains (Gilliland *et al* 1984). Addition of bile to the culture medium resulted in a depressed

growth rate, to varying extents, of all strains tested. Subsequent in-vivo studies of two strains of *L acidophilus* showed that bile tolerance was linked with greater numbers of lactobacilli found in the jejunum of calves.

Many species of gut bacteria possess surface appendages which show mannose-specific adherence to mucosal cells and can be considered as lectins by virtue of their ability to agglutinate cells (Firon *et al* 1984). Because of this linkage, it is feasible that plant lectins may compete with bacteria for binding sites at the brush border. Dietary protein rich in lectins could, therefore, be a disadvantage to the gut colonisation by probiotic organisms.

Significance of faecal counts

Experimenters often use relative changes in faecal counts of lactobacilli and coliforms to assess probiotic efficacy. Faecal measurements are not a satisfactory indicator of the behaviour of these organisms elsewhere in the gastrointestinal tract (Pollmann *et al* 1980; Ward and Nelson 1982). In work with gnotobiotic and conventional pigs, Pollmann *et al* (1980) reported a lack of correlation between counts of lactobacilli or coliforms associated with tissue from the stomach, small intestine, caecum or colon and faecal counts of these organisms. Nevertheless faecal counts of lactobacilli are normally higher than coliforms in healthy pigs and reversed in animals suffering from diarrhoea (Mitchell and Kenworthy 1976; Muralidhara *et al* 1977).

Tissue-damaging reactions to dietary antigens

Evidence of gut inflammatory reactions to antigenic dietary protein has been reported in calves fed milk substitutes and in early weaned piglets (Sissons *et al* 1984; Ratcliffe *et al* 1987). Although pathogenic coliforms are not linked directly with the concomitant diarrhoea, the condition may predispose to their proliferation (Miller *et al* 1985). It is possible that lactic acid bacteria cannot adhere to epithelial cells damaged by adverse immune responses. Thus colonisation studies of probiotic organisms should take account of diet interactions at the mucosal surface.

PROBIOTIC SPECIFICATION

Successful colonisation of the gastrointestinal tract by probiotic bacteria requires the organism to have several attributes. The main features include an ability to adhere to epithelial cells and rapid growth in digesta of continuously varying composition. Studies so far suggest that no single species of bacteria is able to thrive both in the stomach and intestine, although it is possible for some microorganisms to survive in these different environments. It is therefore desirable to select several bacterial species or strains according to their ability to multiply in the proximal or distal regions of the alimentary tract. Most reported experimental protocols have involved daily inoculation of calves and piglets with doses of organisms in the range of 10^9–10^{12} viable counts. There is need for information on the effects of varying the dose and frequency of administration.

Desirable characteristics

In the stomach an organism must adhere to the epithelial cells of the cardiac (non-secretory) region, secrete adequate quantities of lactic acid to reduce the pH of the gastric digesta to less than pH 4·5, withstand physiological concentrations of hydrochloric acid and grow rapidly in digesta containing a variety of intact and partially digested feedstuffs. For successful colonisation of the intestine, probiotic organisms must attach to the brush border and tolerate high concentrations of bile in the upper gut and volatile organic acids in the large intestine. Also, because of the relatively fast transit through the small bowel, the organism should multiply rapidly in intestinal digesta.

Potential probiotic bacteria

Work to date indicates that some strains of *L acidophilus* have probiotic efficacy in the stomach and to a lesser extent in the small intestine, and *S faecium* has features suitable for thriving in the small gut. Phage-resistant strains of *S faecium* may need to be selected to avoid an unknown factor which depresses growth in chickens (Fuller 1986). Another species worthy of consideration is *L lactis*. This organism was selected by Reiter *et al* (1980) for its potential to produce hydrogen peroxide and thereby contribute to the LP system. *L lactis* was also found to prevent coliform proliferation and control diarrhoea in young piglets (Muralidhara *et al* 1977).

CONCLUSIONS

A scant scientific literature provides evidence that probiotic organisms have the potential to protect young farm animals against enteropathogenic disorders. Several mechanisms could account for a beneficial effect, but an ability to colonise epithelial tissue and produce lactic acid would seem to be desirable features for probiotic cultures. There is insufficient information to clarify whether probiotic bacteria can make a positive contribution to digestion of the host. Further work is needed on the modes of action of probiotics, particularly on the possibility that lactic acid bacteria act as agents for promoting immune competence. Information is also required on the efficacy of probiotic cultures formulated with more than one species of bacterium.

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