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Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids

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Probiotics could represent an effective alternative to the use of synthetic substances in nutrition and medicine. The data concerning the efficacy of probiotics are often contradictory. This paper focuses on the enhancement of the efficacy of probiotics by their combination with synergistically acting components of natural origin. Maltodextrins can be obtained by enzymatic hydrolysis of starch and are suitable for consumption. Administration of *Lactobacillus paracasei* together with maltodextrin decreased the number of *Escherichia coli* colonising the jejunal mucosa of gnotobiotic piglets by 1 logarithm compared to the control group. Fructo-oligosaccharides (FOS) are naturally occurring oligosaccharides, mainly of plant origin. *L. paracasei* administered in combination with FOS significantly increased counts of *Lactobacillus* spp., *Bifidobacterium* spp., total anaerobes and total aerobes compared to the control group as well as the *L. paracasei* group. It also significantly decreased *Clostridium* and *Enterobacterium* counts in the faeces of the weanling piglets compared with the control group. Dietary lipids influence the gastrointestinal microbiota and specifically the population of lactic acid bacteria. In gnotobiotic piglets the oral administration of an oil containing polyunsaturated fatty acids (PUFA) significantly increased the number of *L. paracasei* adhering to jejunal mucosa compared to the control group. Our results showed that maltodextrin KMS X-70 and PUFA can be used to enhance the effect of probiotic micro-organisms in the small intestine, and similarly FOS enhance the effect of probiotic micro-organisms in the large intestine.

Probiotics: Maltodextrin: Fructo-oligosaccharides: Polyunsaturated fatty acids

Introduction

According to Fuller (1992), probiotics are biopreparations containing living cells or metabolites of stabilised autochthonous micro-organisms that optimise the colonisation and composition of the gut microflora in both animals and humans and stimulate digestive processes and immunity. For practical purposes it is important that probiotics have effects, such as an inhibitory effect against pathogens, an optimising effect on digestive processes, an immunostimulatory effect, anti-tumour effect and anti-cholesterol action.

The mode of action of probiotics has not been fully explained. The mode of inhibitory action of probiotics against pathogens may be mediated by competition for receptors on the gut mucosa, competition for nutrients, the production of antibacterial substances, and the stimulation of immunity (Piard & Desmazeaud, 1991; Freter, 1992; Perdigon & Alvarez, 1992). Probiotics influence digestive processes by enhancing the population of beneficial micro-organisms, by enhancing microbial enzyme activity and by improving digestibility of foodstuffs and feed utilisation (Burgstaller *et al.* 1984). Optimisation of digestive processes is demonstrated by improved growth and higher weight gains. The anti-tumour activity of probiotics may be realised in three ways: the inhibition of tumour cells; the suppression of bacteria producing beta-glucosidase, beta-glucuronidase, and azoreductase, which catalyse the conversion of procarcinogens to proximal carcinogens; and by the destruction of carcinogens such as nitrosamines and by the suppression of nitroreductase which is involved in their synthesis (Reddy *et al.* 1973; Rowland & Grasso, 1975; Goldin & Gorbach, 1977, 1984). Probiotics influence blood cholesterol level by the inhibition of cholesterol synthesis or by decreasing absorption (Mann, 1977; Zacconi *et al.* 1992).

Abbreviations: FOS, fructo-oligosaccharides; PUFA, polyunsaturated fatty acids.

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Enhancing the efficacy of probiotics

Probiotics as natural bio-regulators help to maintain the balance of the digestive tract ecosystem by a variety of mechanisms and prevent the colonisation of the digestive tract by pathogenic bacteria (Vandenbergh, 1993). In agriculture and veterinary medicine, probiotics may be effectively used particularly in optimising digestive processes, growth stimulation, and in the prevention of digestive tract diseases in young farm animals. The data concerning the efficacy of probiotics in practice are often contradictory. With regard to the application of probiotic lactobacilli to pigs, many authors have reported a growth and stimulatory effect (Baird, 1977; Hale & Newton, 1979; Pollmann *et al.* 1980; Nousiainen & Setälä, 1993). However, some authors did not observe growth improvement with the administration of probiotics. The data concerning the efficacy of probiotics in the prevention of diarrhoeal diseases in young animals are also contradictory. The effect of lactobacilli and bifidobacteria against diarrhoea in pigs was confirmed by several reports (Hale & Newton 1979; Kimura *et al.* 1983; Maeng *et al.* 1989; Depta *et al.* 1998; Bomba *et al.* 1998). However, other authors (De Cupere *et al*. 1992; Bekaert *et al.* 1996) have not confirmed this effect.

The efficacy of probiotics under different conditions may be due to the probiotic preparation itself or may be caused by other factors. Variability of the data may be due to: low survival rate of strains, stability of the strain, the use of a non-specific strain relative to the host, low dose and frequency of administration, interactions with some medicines, health and nutritional status of the animal and the effect of age, stress, genetics and type differences of animals. Research experience points to the fact that probiotics are most effective in animals during microflora development or when microflora stability is impaired (Stavric & Kornegay, 1995). A probiotic strain should be non-pathogenic and be able to tolerate the conditions of the digestive tract and adhere in high numbers to the digestive tract mucosa; it should be able to maintain high viability during processing, lyophilisation, and storage, re-vitalise quickly in the digestive tract; it should be able to produce inhibitory substances against pathogens and stimulate the immune system (Chesson, 1993). Some of the above-mentioned criteria for the selection of micro-organisms for probiotic purposes can be tested *in vitro*, but most of them must be verified *in vivo*. Some properties of micro-organisms observed under laboratory conditions have not been confirmed in trials with animals (Chateau *et al.* 1993; Bomba *et al.* 1996).

In order to enhance the efficacy of probiotics, it is necessary to obtain important knowledge of the mechanisms mediating their effect in the digestive tract (Stavric & Korgenay, 1995). The anti-bacterial effect of each probiotic micro-organism or its beneficial effect on the macro-organism may be mediated by one or a number of mechanisms that may be expressed at different degrees of intensity. This indicates that it is necessary to study thoroughly the mode of action of each probiotic micro-organism so that the multi-factorial nature of the mechanism can be explained. The efficacy of probiotics may be enhanced by the following methods:

- the selection of more efficient strains of micro-organism
- genetic manipulation
- the combination of a number of probiotic strains
- the combination of probiotics and synergistically acting components.

The combination of probiotics with synergistically acting components of natural origin seems to be a way of enhancing the efficacy of probiotic preparations from the practical point of view. It seems that a number of suitable components may be used to potentiate the effect of probiotics, such as oligosaccharides, phyto-components, nutrients and growth factors, proteins, polyunsaturated fatty acids (PUFA), organic acids and bacterial metabolites (Pollmann *et al.* 1980; Gálfi & Bokori, 1990; Gibson & Roberfroid, 1995; Yadava *et al.* 1995).

Probiotics and maltodextrins

We have found that under *in vitro* conditions *Lactobacillus paracasei* utilised KMS X-70 maltodextrins. However, the pathogenic *Escherichia coli* 08: K88 grew poorly in its presence. *L. paracasei* inhibited the growth of pathogenic *E. coli* 08: K88 strain in the presence of KMS X-70 maltodextrin. These results suggest that under *in vitro* conditions, KMS X-70 maltodextrin may induce the growth of *L. paracasei* as well as the colonisation of the digestive tract under *in vivo* conditions.

We investigated the influence of administration of *L. paracasei* and maltodextrin KMS X-70 (JEP CEREPA, Červená Řečice, Czech Republic) on *E. coli* adhesion in the gastrointestinal tract of gnotobiotic piglets. The administration of *L. paracasei* alone had no inhibitory effect on the adhesion of *E. coli* to the jejunal mucosa of gnotobiotic piglets while *L. paracasei* administered together with maltodextrin decreased the number of *E. coli* colonising the jejunal mucosa of gnotobiotic piglets by 1 logarithm ($4{\cdot}95\log 10/\mathrm{cm}^2$) in comparison to the control group ($5{\cdot}96\log 10/\mathrm{cm}^2$, Fig. 1). Maltodextrin KMS X-70 stimulated the inhibitory effect of *L. paracasei* on the adhesion of *E. coli* to the jejunal mucosa of gnotobiotic piglets.

Probiotics and fructo-oligosaccharides

Fructo-oligosaccharides (FOS) are naturally occurring oligosaccharides, mainly of plant origin. They have been shown to be resistant to endogenous glycolytic enzymes of the host and to pass unaltered to the colon (Oku *et al.* 1984). FOS can significantly modulate the colonic microbiota by increasing the number of specific bacteria and thus changing the composition of the microbiota.

The concept of synbiotics (a mixture of probiotics and oligosaccharides) has recently been proposed to characterise health-enhancing foods and supplements used as functional food ingredients in humans (Gibson & Roberfroid, 1995; Kontula *et al.* 1998). With a combination of both a probiotic and an oligosaccharide, the benefits include improved survival of the probiotic bacteria during passage through the upper intestinal tract and a more efficient

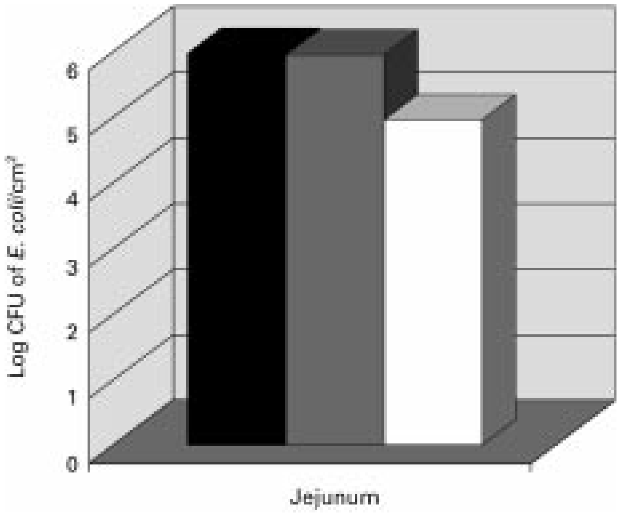

Fig. 1. The numbers of *Escherichia coli* 08: K88 adhering to the jejunal mucosa in 7-day-old gnotobiotic pigs after administration of *Lactobacillus paracasei* and maltodextrin KMS X-70. (■), *E. coli* 08: K88; (■), *L. paracasei* + *E. coli* 08: K88; (□), *L. paracasei* + maltodextrin KMS X-70 + *E. coli* 08: K88.

implantation in the colonic microbiota, together with a stimulating effect of the oligosaccharide on the growth and/or activities of both the exogenous (probiotic) and endogenous bacteria (Roberfroid, 1998).

We examined the effect of the administration of *L. paracasei* and a mixture of *L. paracasei* and FOS on faecal bacterial counts of weanling pigs under field conditions.

Numbers of individual bacterial populations found in both experimental and control animals are presented in Table 1. Significantly higher counts of *Lactobacillus* spp. ($P<0{\cdot}01$), *Bifidobacterium* spp. ($P<0{\cdot}05$), total anaerobes ($P<0{\cdot}05$) and total aerobes ($P<0{\cdot}05$) were found in faeces of experimental animals receiving the mixture of *L. paracasei* and FOS (Raftilose P95, Raffinerie Tirlemontoise, Tienen, Belgium) compared with the controls. Moreover, significantly higher numbers of anaerobes ($P<0{\cdot}05$), total aerobes ($P<0{\cdot}05$), *Bifidobacterium* ($P<0{\cdot}05$) and *Lactobacillus* ($P<0{\cdot}05$) counts were found compared to the *L. paracasei* group. Compared to the controls, significant decreases in *Clostridium* ($P<0{\cdot}05$) and *Enterobacterium* ($P<0{\cdot}01$) counts were also observed as well as an insignificant decrease in coliform counts. In addition, *Enterococcus* counts were significantly reduced ($P<0{\cdot}001$) compared to both the control group and the *L. paracasei* group. In faeces of experimental animals receiving *L. paracasei*, significant decreases in *Clostridium* ($P<0{\cdot}05$) and *Enterobacterium* ($P<0{\cdot}05$) counts as compared with the controls were recorded. Coliform counts were lower by 0·5 log compared with controls. This difference, however, was not significant due to the great individual variability in the data. *Lactobacillus*, *Enterococcus* and total anaerobes counts were identical in both groups. A non-significant increase in total aerobes in the experimental group was recorded and there was a non-significant decrease in *Bifidobacterium* spp. as compared to the control group. The results of this study point to a synergistic effect of the *L. paracasei* and FOS combination on faecal microflora of weaned pigs.

Probiotic and polyunsaturated fatty acids

Competition for receptors on the gut mucosa is one mechanism of inhibitory action of probiotics against pathogens in the digestive tract of animals (Stavric *et al.* 1987). Improvement in the colonisation of the intestinal mucosa by probiotic bacteria enhances the inhibitory effect of probiotics upon the adhesion of pathogens. It was demonstrated that dietary lipid influences the gastrointestinal microbiota and especially the population level of lactic acid bacteria (Ringo *et al.* 1998). According to Kankaanpää *et al.* (2001), higher concentrations of PUFA inhibited the growth and mucus adhesion of selected lactobacilli, whilst growth and mucus adhesion of *Lactobacillus casei* Shirota was promoted by low concentrations of γ-linolenic acid and arachidonic acid, respectively. PUFA also altered bacterial adhesion sites on Caco-2 cells. It is suggested that dietary PUFA affects the attachment sites for the gastrointestinal microbiota, possibly by modifying the fatty acid composition of the intestinal wall.

We studied the effect of administration of PUFA on the

Table 1. Composition of faecal microflora in weanling pigs receiving *Lactobacillus paracasei* and mixture of *L. paracasei* and fructo-oligosaccharides (FOS)

(Mean values with their standard errors of log bacteria counts per gram of wet faeces for seven determinations)

	Control		*L paracasei*		*L. paracasei* + FOS	
Organism	Mean	SE	Mean	SE	Mean	SE
Total anaerobes	9·8	0·2	9·8	0·3	10·2	0·2†*, ‡*
Total aerobes	8·0	0·5	8·3	0·2	9·3	0·7†*, ‡*
Bifidobacterium spp.	7·5	0·3	7·1	0·7	8·2	0·3†*, ‡*
Lactobacillus spp.	9·9	0·1	9·9	0·3	10·3	0·1†**, ‡*
Enterococcus spp.	9·3	0·1	9·3	0·3	8·2	0·2†***, ‡***
Clostridium spp.	8·1	0·1	7·4	0·4†*	7·7	0·3†*
Enterobacteriaceae	7·9	0·4	6·5	0·9†*	5·9	0·9†**
Coliforms	6·8	0·7	6·3	0·7	5·8	0·7

† Significantly different from control group.
‡ Significantly different from *L. paracasei* group.
*$P<0{\cdot}05$; **$P<0{\cdot}01$; ***$P<0{\cdot}001$.

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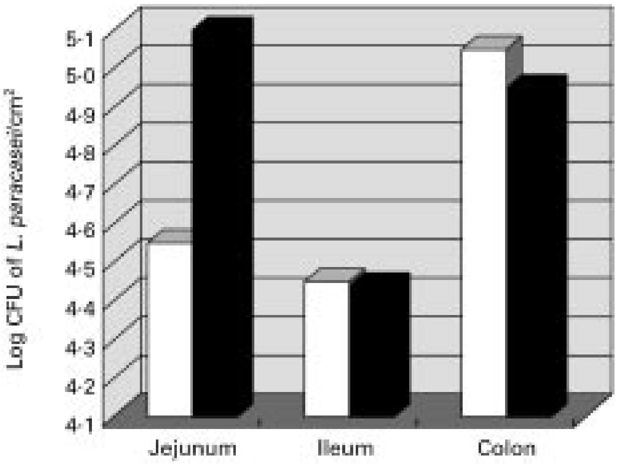

Fig. 2. *Lactobacillus paracasei* colonisation of the jejunal, ileal and colonic mucosa of gnotobiotic piglets after administration of polyunsaturated fatty acids (PUFA) or NaCl. (□), *L. paracasei* + 0·7 % NaCl; (■), *L. paracasei* + PUFA.

adhesion of *L. paracasei* to the intestinal mucosa in the gnotobiotic piglets. The number of *L. paracasei* adhering to jejunal mucosa in the gnotobiotic piglets orally administered an oil blend (Seal oil, Star Enterprises, Saint John, Newfoundland, Canada) containing 0·1 g total *n*-6 PUFA, 1·0 g total *n*-3 PUFA, 2·6 g total monounsaturated fatty acids, 0·9 g total saturated fatty acids and 0·005 g cholesterol was significantly higher ($P<0·05$, 5·10 log 10/cm^2) in comparison with the control group (4·55 log 10/cm^2; Fig. 2). Administration of the PUFA affected the adhesion of *L. paracasei* to the jejunal mucosa of gnotobiotic piglets. The stimulatory effect of PUFA upon adhesion of lactobacilli could be used for enhancing the effectiveness of probiotics in inhibiting digestive tract pathogens.

Conclusions

Future research should be aimed at the selection of strains with strong probiotic effects, which will comply with specific criteria of selection. It will be important to search for ways to potentiate the efficacy of probiotic micro-organisms in all regions of the digestive tract. In addition to prebiotics, which potentiate the effect of probiotics in the colon, there should be components that, in combination with probiotic preparations, will ensure their efficacy in the small intestine also. Our results showed that maltodextrin KMS X-70 and PUFA can be used for potentiating the probiotic effect in the small intestine, and FOS can be used for potentiating the probiotic effect in the large intestine. It has been suggested that their combination may result in potentiation of the probiotic effect in all sections of the digestive tract but this hypothesis needs further research.

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