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 microorganisms, 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 microorganisms for probiotic purposes can be tested *in vitro*, but most of them must be verified *in vivo*. Some properties of microorganisms 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 & Kornegay, 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; Gáli & 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 CEREP, 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 (4.95 log 10/cm²) in comparison to the control group (5.96 log 10/cm², 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
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_ < 0.01), _Bifidobacterium_ spp. (_P_ < 0.05), total anaerobes (_P_ < 0.05) and total aerobes (_P_ < 0.05) 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_ < 0.05), total aerobes (_P_ < 0.05), _Bifidobacterium_ (_P_ < 0.05) and _Lactobacillus_ (_P_ < 0.05) counts were found compared to the _L. paracasei_ group. Compared to the controls, significant decreases in _Clostridium_ (_P_ < 0.05) and _Enterobacterium_ (_P_ < 0.001) counts were also observed as well as an insignificant decrease in coliform counts. In addition, _Enterococcus_ counts were significantly reduced (_P_ < 0.001) compared to both the control group and the _L. paracasei_ group. In faeces of experimental animals receiving _L. paracasei_, significant decreases in _Clostridium_ (_P_ < 0.05) and _Enterobacterium_ (_P_ < 0.05) 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.

### Table 1. Composition of faecal microflora in weanling pigs receiving _Lactobacillus paracasei_ and mixture of _L. paracasei_ and fructo-oligosaccharides (FOS)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Control</th>
<th><em>L. paracasei</em></th>
<th><em>L. paracasei</em> + FOS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Total anaerobes</td>
<td>9.8</td>
<td>0.2</td>
<td>9.8</td>
</tr>
<tr>
<td>Total aerobes</td>
<td>8.0</td>
<td>0.5</td>
<td>8.3</td>
</tr>
<tr>
<td>Bifidobacterium spp.</td>
<td>7.5</td>
<td>0.3</td>
<td>7.1</td>
</tr>
<tr>
<td><em>Lactobacillus</em> spp.</td>
<td>9.9</td>
<td>0.1</td>
<td>9.9</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp.</td>
<td>9.3</td>
<td>0.1</td>
<td>9.3</td>
</tr>
<tr>
<td><em>Clostridium</em> spp.</td>
<td>8.1</td>
<td>0.1</td>
<td>7.4</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>7.9</td>
<td>0.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Coliforms</td>
<td>6.8</td>
<td>0.7</td>
<td>6.3</td>
</tr>
</tbody>
</table>

† Significantly different from control group.
‡ Significantly different from _L. paracasei_ group.
* _P_ < 0.05; ** _P_ < 0.01; *** _P_ < 0.001.

### 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 Kankaانpää et al. (2001), higher concentrations of PUFA inhibited the growth and mucus adhesion of selected lactobacilli, whilst growth and mucus adhesion of _Lactobacillus casei_ Shirata 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...
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 adminis-
terated 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 choles-
terol was significantly higher ($P<0·05, 5·10^{10}\text{cm}^{-2}$)
in comparison with the control group ($4·55\times10^{10}\text{cm}^{-2}$;
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 lacto-
cabilli 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 probiotics, which potentiate the effect of pro-
biotics 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 com-
bination may result in potentiation of the probiotic effect
in all sections of the digestive tract but this hypothesis needs
further research.

References

49, 11.
Bekaert H, Moermans R & Eckhout W (1996) Influence d’une
culture de levure vivante (Levucell SB2) dans un aliment
pour porcelets sevrés sur les performances zootechniques et
sur la fréquence des diarrhées (Influence of the live culture
of yeasts in feed on the performance and incidence of diarrhea
Bomba A, Gancarcikova S, Nemcová R, Herich R, Kaštel’ R,
effect of lactic acid bacteria on intestinal metabolism and meta-

tabolic profile of gnotobiotic pigs. Deutsche Tierärztliche
Wochenschrift 105, 384–389.
Bomba A, Kaštel’ R, Gancarčíková S, Nemcová R, Herich R &
Čižek M (1996) The effect of Lactobacilli inoculation on
organic acid levels in the mucosal film and the small intestine
contents in gnotobiotic pigs. Berliner und Münchener Tierärzt-
lische Wochenschrift 109, 428–430.
Burgstaller G, Ferstl R & Apls H (1984) Zum Zusatz von Milch-
säurebakterien (Streptococcus faecium SF-68) im Milchaus-
taußchüttermittel für Mastkalber (The addition of lactic acid
bacteria to a milk replacer for calf feeding). Zuchtungskunde
56, 156–162.
Chateau N, Castellanos I & Deschamps AM (1993) Distribution
of pathogen inhibition in the Lactobacillus isolates of a com-
mercial probiotic consortium. Journal of Applied Bacteriology
74, 36–40.
Chesson A (1993) Probiotics and other intestinal mediators. In
Principles of Pig Science, pp. 197–214 [DJA Cole, J-MA
Wisman and MA Varley, editors]. Loughborough: Nottingham
University Press.
De Cupere F, Deprez P, Demeulenaere D & Muylle E (1992)
Evaluation of the effect of 3 probiotics on experimental Escher-
ichia coli enterotoxaemia in weaned piglets. Journal of Veteri-
nary Medicine B 39, 277–284.
Depa A, Rychlik R, Nieradka R, Rotkiewicz T, Kujawa K,
alimentary tract colonization with Lactobacillus sp. strains on
chosen metabolic profile indices in piglets. Polish Journal of
Veterinary Science 1–2, 3–7.
Freter R (1992) Factors affecting the microecology of the gut.
In Probiotics: The Scientific Basis, pp. 111–114 [R Fuller, editor].
London: Chapman and Hall.
Fuller R (1992) The effect of probiotics on the gut microbiology
of farm animals. In The Lactic Acid Bacteria, pp. 171–192
Gáffi P & Bokori J (1990) Feeding trial in pigs with a diet con-
taining sodium n-butyrate. Acta Veterinaria Hungarica
38, 3–17.
Gibson GR & Roberfroid MB (1995) Dietary modulation of the
human colonic microbiota: introducing the concept of pre-
Goldin BR & Gorbach SL (1977) Alterations in fecal microflora
enzymes related to diet, age, lactobacillus supplements and
Goldin BR & Gorbach SL (1984) The effect of milk and lacto-
cabillus feeding on human intestinal bacterial enzyme activity.
Hale OM & Newton GL (1979) Effects of a non-viable lacto-
cabillus species fermentation product on performance of pigs.
Kankaanpää PE, Salminen SJ, Isolauri E & Lee YK (2001) The
influence of polyunsaturated fatty acids on probiotic growth
application of dried bifidobacteria preparation to scurrying
animals. Bifidobacteria and Microflora 2, 41–55.
Kontula P, Jaskari J, Nollet L, De Smet I, von Wright A, Poutanen K
of the human intestinal microbial ecosystem by a probiotic

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


