Veterinary Research Communications, 31 (2007) 791–800
DOI: 10.1007/s11259-007-0048-x

Effects of the Administration of Lactobacilli, Maltodextrins and Fructooligosaccharides upon the Adhesion of *E. coli* O8:K88 to the Intestinal Mucosa and Organic Acid Levels in the Gut Contents of Piglets

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ABSTRACT

The influence of the administration of *Lactobacillus plantarum*, maltodextrin Maldex 150 and Raftifeed IPX fructooligosaccharides on the inhibition of adhesion of *E. coli* O8:K88 to the mucosa of the jejunum, ileum and colon as well as on the organic acid levels was investigated in 33 conventional piglets. The counts of *E. coli* K88 adhering to the jejunal mucosa were significantly decreased ($p < 0.05$) in *Lact. plantarum* + Maldex 150 and *Lact. plantarum* + Maldex 150 + Raftifeed IPX groups. The counts of *E. coli* K88 adhering to the colonic mucosa of *Lact. plantarum* + Maldex 150 + Raftifeed IPX and *Lact. plantarum* + Raftifeed IPX groups were significantly lower ($p < 0.05$) than in *Lact. plantarum* and *Lact. plantarum* + Maldex 150 animals. The acetic acid levels in the ileum and colon of the *Lact. plantarum* + Maldex 150 + Raftifeed IPX group and *Lact. plantarum* + Raftifeed IPX group were significantly higher ($p < 0.05$) than in the *Lact. plantarum* and *Lact. plantarum* + Maldex 150 group. The combination of *Lact. plantarum*, maltodextrin Maldex 150 and Raftifeed IPX proved to be the most effective one to inhibit the counts of *E. coli* O8:K88 adhering to the intestinal mucosa of the jejunum and colon of conventional piglets.

Keywords: adhesion, fructooligosaccharides, intestine, lactobacilli, maltodextrins, piglets

Abbreviations: cfu, colony-forming units; *Lact. plantarum*, *Lactobacillus plantarum*; MRS, de Man – Rogosa – Sharpe broth; PBS, phosphate-buffered saline; TLC, thin-layer chromatography

INTRODUCTION

In agriculture and veterinary medicine, probiotics may be used to optimize digestive processes, to stimulate growth and to protect the digestive tract of young farm animals from diseases (Nousiainen and Setälä, 1993). Data concerning the efficacy of probiotics in the prevention of diarrhoeic diseases in young animals in practice are often contradictory. Although Kimura and colleagues (1983), Maeng and colleagues (1989), Depta and colleagues (1998) and Bomba and colleagues (1998) have stated that lactobacilli and bifidobacteria positively affect diarrhoea in pigs, several other authors (De Cupere *et al.*, 1992; Bekaert *et al.*, 1996) did not confirm such effects. The variation in efficacy of probiotics under different conditions may be attributable to the probiotic preparation itself or may be caused by external conditions.

From the practical point of view, the combination of probiotics with synergistic components of natural origin seems to be the best way of enhancing the efficacy of probiotic preparations. It seems that a number of suitable components such as prebiotics, phytocomponents, nutrients and growth factors, proteins, polyunsaturated fatty acids, organic acids and bacterial metabolites may be used to potentiate the effect of probiotics (Bailey *et al.*, 1991, Yadava *et al.*, 1995; Bury *et al.*, 1998; Bomba *et al.*, 2002, 2003).

The concept of 'synbiotics' (mixtures of probiotics and prebiotics) has been proposed to characterize health-enhancing foods and supplements used as functional food ingredients in humans (Gibson and Roberfroid, 1995). According to Crittenden (1999), the prebiotic approach for increasing beneficial bacteria in the colon potentially provides some advantages over the probiotic strategy. Specifically, consumed probiotic bacteria must survive transit through the hostile conditions in the stomach and then adapt quickly to their new environment. In contrast, prebiotics offer the potential to increase not only the numbers of beneficial bacteria but also their metabolic activity through the supply of fermentable substrate. The increase in the metabolic activity of autochthonous or allochthonous (probiotic) microorganisms is fundamental to many of the currently proposed mechanisms of health promotion by prebiotics.

The aim of the present study was to compare the effects of the administration of *Lactobacillus plantarum,* maltodextrin Maldex 150 and Raftifeed IPX fructooligosaccharides upon the inhibition of the adhesion of *E. coli* O8:K88 to the mucosa of the jejunum, ileum and colon and upon organic acid levels in the gut contents of conventional suckling pigs.

MATERIALS AND METHODS

Animals and nutrition

In a 7-day study, 33 conventional healthy suckling piglets of average live weight at birth of 1.2 kg were used. The sows of Large White breed originated from Perin farm, Slovak Republic. The sows and piglets were housed in an environmentally controlled building. The piglets were provided access *ad libitum* to maternal milk.

The experimental protocol was approved by the State Veterinary and Food Administration of the Slovak Republic.

Substrates and bacterial strains

Maltodextrin Maldex 150 (Amylum Boleráz, Slovak Republic) is a partially hydrolysed starch with a dextrose equivalent of 15 according to the manufacturer. The fructooligosaccharide Raftifeed IPX (Orafti, Raffinerie Tirlemontoise, Tienen, Belgium) contained 70% inulin and 30% oligofructose.

The *Lactobacillus* strain was selected from the gut contents of healthy suckling piglets and identified as *Lact. plantarum* using the API 50 CHL system (BioMerieux, Marcy–L'Etoile, France). The strain was characterized by strong adherence to the epithelial cells

from the porcine intestine, by inhibitory activity against *Escherichia coli* O8:K88ab:H9 in *in vitro* conditions, and by production of hydrogen peroxide (Nemcová *et al.*, 1997). The *Lact. plantarum* strain was able ferment Maldex 150 and Raftifeed IPX as a single-carbon source in a minimal medium (Nemcová, 1998). For this study, the strain was grown in de Man, Rogosa and Sharpe broth (MRS; Merck, Darmstadt, Germany) for 18 h at 37 °C. The optical density at 640 nm of the bacterial culture was adjusted to 0.5 to give approximately 1×10^8 colony forming units (cfu)/ml (Specol EK, Carl Zeiss, Jena, Germany). Additionally, counts for the bacterial culture used were established by plate counting on MRS agar (Merck) after preparation of the inoculum.

The *E. coli* O8:K88ab:H9 strain without enterotoxin production was obtained from the Laboratory of Immunology and Gnotobiology, Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic. Overnight culture of *E. coli* (1 ml)was inoculated into 50 ml Trypticase soy broth (Oxoid Unipath, Ltd., Basingstoke, UK) and cultivated at 37 °C in a water bath shaker (JULABO SW 2C, Labor Technic GMBH Selbach, Germany) for approximately 2 h to optical density 0.5 at 640 nm (corresponded to 1×10^8 cfu/ml). Subsequently, the bacterial culture was diluted in isotonic saline solution to obtain a final concentration of 1×10^5 cfu/ml. Additionally, counts for the bacterial culture used were established by plate counting on MacConkey agar (Oxoid Unipath) after preparation of the inoculum.

Inoculation of pigs and administration of maltodextrin Maldex 150 and Raftifeed IPX

The animals were divided into 4 groups, each group from one sow:

Group L (*Lact. plantarum*) 8 piglets
Group LM (*Lact. plantarum* + Maldex 150) 8 piglets
Group LF (*Lact. plantarum* + Raftifeed IPX) 8 piglets
Group LMF (*Lact. plantarum* + Maldex 150 + Raftifeed IPX) 9 piglets

In the course of the experiment, the piglets of all groups were inoculated orally each day with 2 ml of *Lact. plantarum* (1×10^8 cfu/ml). The inoculum was applied for the first time on the day of birth of the piglets. In addition, maltodextrin Maldex 150 was administered to the animals of groups LM and LMF, while Raftifeed IPX was administered to those of groups LF and LMF. Maldex 150 and Raftifeed IPX were administered orally four times a day at a dose of 0.3 g. At the age of 5 days, the piglets of all groups were challenged orally with 2 ml of *E. coli* O8:K88ab:H9 (1×10^5 cfu/ml).

Biological material and chemical analyses

The pigs of all groups were sacrificed with T61 (Intervet International B.V. Boxmeer, The Netherlands) intracardially at the age of 7 days, i.e. 2 days after *E. coli* inoculation. No clinical signs of disease were observed and the animals were in good condition of health.

Segments of mid-jejunum, distal ileum and spiral colon (10 cm in length and 1 cm in width: area 10 cm^2) were excised immediately after slaughter and rinsed with 20 ml

0.15 mol/L sterile phosphate-buffered saline (PBS, pH 7, 2) to remove lumen contents. Then the tissues were washed 3 times with 0.15 mol/L PBS and homogenized (Stomacher Lab Blender 80, Seward Medical Limited, London, UK). A series of 10-fold dilutions (10^{-2} to 10^{-9}) were made in isotonic saline solution. From appropriate dilutions, 0.1 ml aliquots were spread onto MacConkey agar. The plates were incubated for 24 h at 37 °C. All the colonies on the final dilution plate were tested by slide agglutination with K88ab antiserum (Imuna Šarišké Michaany, Slovak Republic). The positive K88ab lactose-fermenting colonies were counted and expressed as the $\log_{10}$ of cfu/cm^{-2} of intestinal mucosa.

Contents (1 g) of jejunum, ileum and colon were placed in sterile polyethylene Stomacher Lab Blender bag (Seward Medical) with 9 ml of sterile anaerobic diluent (0.4 g $NaHCO_3$, 0.05 g L-cysteine-HCl, 1 ml resazurin (0.1%), 7.5 ml mineral solution I (0.6% K_2HPO_4), 7.5 ml mineral solution II (1.2% NaCl, 1.2% $(NH_4)_2SO_4$, 0.6% KH_2PO_4, 0.12% $CaCl_2$, 0.25% $MgSO_4$) and 84 ml distilled water, pH 6.8) and stomached for 2 min under a CO_2 atmosphere. A series of 10-fold dilutions (10^{-2} to 10^{-9}) were made in the same diluents. From appropriate dilutions, 0.1 ml aliquots were spread onto MRS agar for total lactobacilli. The plates were incubated at 37 °C for 48 h under anaerobiosis (Gas Pak Plus, BBL, Microbiology systems, Cockeysville, USA). The viable counts are expressed as the $\log_{10}$ of cfu/g of content.

The contents of jejunum, ileum and colon were sampled and stored at −80 °C until analysis for organic acid concentrations and chromatographic separation. Intestinal contents (1 g) were diluted in 50 ml deionized water, homogenized (Stomacher Lab Blender 80, Seward Medical Limited, London, UK) and filtered through filter paper. A sample of 30 µl was used for the analysis of organic acids. Lactic and acetic acid concentrations in the intestinal contents were determined by capillary isotachophoresis using a capillary isotachophoresis analyser (Radioecological Institute, Košice, Slovak Republic). As conducting and finishing electrolytes, 0.001 mmol/L hydrochloric acid (pH 4.25) and 5 mmol/L caproic acid (pH 4.5) were used, respectively.

Separation of Maldex 150 and Raftifeed IPX samples was carried out by the rapid and simple thin-layer chromatographic method (TLC) with postchromatographic derivation of chromatograms. Chromatographic separation was performed on Silicagel-precoated glass plates (10 × 10 cm; Merck, Darmstadt, Germany) with butanol–ethanol–water (5:3:2, v/v/v) as the mobile phase. Prior to development in the mobile phase, the layer was impregnated with 0.02 mol/L sodium acetate. Postchromatographic derivatization of chromatograms was accomplished with a diphenylamine–aniline–phosphoric acid reagent. After impregnation, the layers were oven-dried at 50 °C for 5 min. Volumes of 0.2 µl of the preprocessed standard solutions of Maldex 150, Raftifeed IPX and the biological samples were spotted on the start of the plates manually using a microsyringe. Chromatographic separation was accomplished in a vertical trough chamber by saturation of solvent vapours. A one-dimensional development of the chromatograms was used. After development, the plates were dried for 15 min in a flow of warm air, and then immersed for 5 s in a dipping solution (aniline–diphenylamine–phosphoric acid). After drying of the plates at room temperature (10 min), the colour was produced by heating at 120 °C for 20 min. Densitometric analysis of the chromatograms was performed at $\lambda = 370$ nm in the linear scan mode.

Statistical analysis

Statistical evaluation of the results was performed by Kruskal–Wallis one-way analysis. The significance of differences between mean values was calculated by the Student–Newman–Keuls method.

RESULTS

Figure 1 gives the counts of *E. coli* adhering to the mucosa of the jejunum, ileum and colon. The counts of *E. coli* K88 adhering to the jejunal mucosa of 7-day-old pigs reached 6.63 $\pm$ 0.882 $\log_{10}$ cm^{-2} in group *Lact. plantarum*; 5.11 $\pm$0.534 $\log_{10}$ cm^{-2} in group *Lact. plantarum* + Maldex 150; 6.30 $\pm$ 0.518 $\log_{10}$ cm^{-2} in group *Lact. plantarum* + Raftifeed IPX; and 4.47 $\pm$ 0.849 $\log_{10}$ cm^{-2} in group *Lact. plantarum* + Maldex 150 + Raftifeed IPX. In the ileal mucosa of 7-day-old pigs, *E. coli* K88 counts of 7.16 $\pm$ 0.647 $\log_{10}$ cm^{-2} were found in group *Lact. plantarum*; 6.97 $\pm$ 0.463 $\log_{10}$ cm^{-2} in group *Lact. plantarum* + Maldex 150; 6.84 $\pm$ 0.373 $\log_{10}$ cm^{-2} in group *Lact. plantarum* + Raftifeed IPX; and 6.84 $\pm$ 0.373 $\log_{10}$ cm^{-2} in group *Lact. plantarum* + Maldex 150 + Raftifeed IPX. The counts of *E. coli K88* adhering to the colonic mucosa of 7-day-old pigs amounted to 7.14 $\pm$ 0.387 $\log_{10}$ cm^{-2} in group *Lact. plantarum*; 7.20 $\pm$ 0.513 $\log_{10}$ cm^{-2} in group *Lact. plantarum* + Maldex 150; 6.60 $\pm$ 0.265 $\log_{10}$ cm^{-2} in group *Lact. plantarum* + Raftifeed IPX; and 6.20 $\pm$ 0.732 $\log_{10}$ cm^{-2} in group *Lact. plantarum* + Maldex 150 + Raftifeed IPX. When compared to group *Lact. plantarum*, the counts of *E. coli* K88 adhering to the

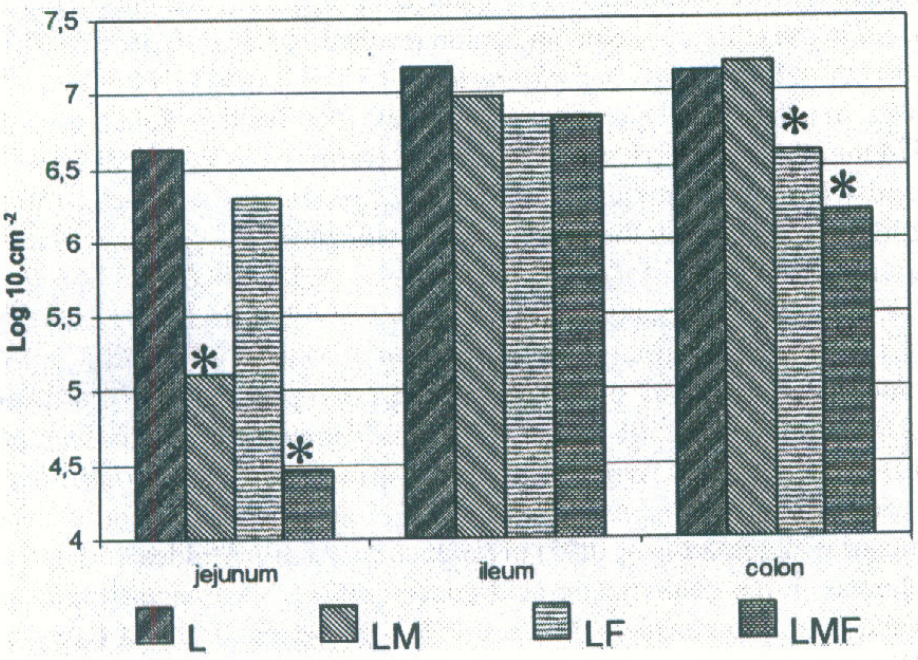

Figure 1. The counts of *E. coli* O8:K88 adhering to the intestinal mucosa in 7-day-old conventional pigs. L, *Lact. plantarum* ($n = 8$); LM, *Lact. plantarum* + Maldex 150 ($n = 8$); LF, *Lact. plantarum* + Raftifeed IPX ($n = 8$); LMF, *Lact. plantarum* + Maldex 150 + Raftifeed IPX ($n = 9$)

TABLE I
The counts of total lactobacilli (log10 g^{-1}) in the gut content in 7-day-old conventional pigs

Segment of the gut	L	LM	LF	LMF
Jejunum	6.13 ± 1.595	5.98 ± 0.589	6.56 ± 0.489	6.65 ± 0.407
Ileum	6.96 ± 0.438	6.58 ± 0.604	7.36 ± 0.360	7.40 ± 0.696
Colon	7.45 ± 0.990	7.29 ± 0.457	7.97 ± 0.408	8.02 ± 0.688

L, *Lact. plantarum* ($n = 8$); LM, *Lact. plantarum* + Maldex 150 ($n = 8$); LF, *Lact. plantarum* + Raftifeed IPX ($n = 8$); LMF, *Lact. plantarum* + Maldex 150 + Raftifeed IPX ($n = 9$)

jejunal mucosa were significantly decreased ($p < 0.05$) in *Lact. plantarum* + Maldex 150 and *Lact. plantarum* + Maldex 150 + Raftifeed IPX animals. Comparison revealed the counts of *E. coli* K88 adhering to the colonic mucosa of *Lact. plantarum* + Maldex 150 + Raftifeed IPX and *Lact. plantarum* + Raftifeed IPX animals to be significantly lower ($p < 0.05$) than in *Lact. plantarum* and *Lact. plantarum* + Maldex 150 animals.

Table I shows counts of total lactobacilli in the contents of jejunum, ileum and colon. In group *Lact. plantarum* + Maldex 150, administration of maltodextrin did not influence the total counts of lactobacilli in the gut content. In the contents of the jejunum, ileum and colon in animals receiving *Lact. plantarum* + Maldex 150 + Raftifeed IPX and *Lact. plantarum* + Raftifeed IPX, higher total lactobacilli counts were found than in the *Lact. plantarum* group. This difference, however, was nonsignificant owing to the great variance of values in individual groups.

Figure 2 depicts the concentrations of organic acids in the gut contents of pigs. Lactic acid concentrations in the jejunum, ileum and colon reached 16.03 ± 10.383, 10.31 ± 9.328 and 2.98 ± 1.540 mmol/L in group *Lact. plantarum*; 11.17 ± 3.484, 12.43 ± 10.757 and 8.97 ± 6.761 mmol/L in group *Lact. plantarum* + Maldex 150; 19.31 ± 8.422, 6.95 ± 4.952 and 4.43 ± 1.574 mmol/L in group *Lact. plantarum* + Raftifeed IPX; and 19.20 ± 7.092, 35.86 ± 23.854 and 4.18 ± 1.147 mmol/L in group *Lact. plantarum* + Maldex 150 + Raftifeed IPX. The differences between the groups were nonsignificant. Acetic acid concentrations in the jejunum, ileum and colon were respectively 18.58 ± 8.025, 17.86 ± 13.770 and 23.64 ± 7.478 mmol/L in group *Lact. plantarum*; 21.02 ± 16.673, 13.60 ± 11.637 and 26.93 ± 14.116 mmol/L in group *Lact. plantarum* + Maldex 150; 27.03 ± 13.113, 15.34 ± 13.270 and 53.62 ± 16.683 mmol/L in group *Lact. plantarum* + Raftifeed IPX; and 26.56 ± 12.046, 51.43 ± 21.805 and 62.45 ± 8.017 mmol/L in group *Lact. plantarum* + Maldex 150 + Raftifeed IPX. When compared to groups *Lact. plantarum*, *Lact. plantarum* + Maldex 150 and *Lact. plantarum* + Raftifeed IPX, acetic acid concentrations in the ileum were significantly increased ($p < 0.05$) in *Lact. plantarum* + Maldex 150 + Raftifeed IPX animals, whereas in the colon acetic acid concentrations were significantly increased ($p < 0.05$) in the *Lact. plantarum* + Maldex 150 + Raftifeed IPX and *Lact. plantarum* + Raftifeed IPX group when compared to groups *Lact. plantarum* and *Lact. plantarum* + Maldex 150.

Intra-group comparison of organic acid concentrations in the individual gut segments revealed maximum ($p < 0.05$) lactic acid concentrations in the jejunal contents of *Lact.*

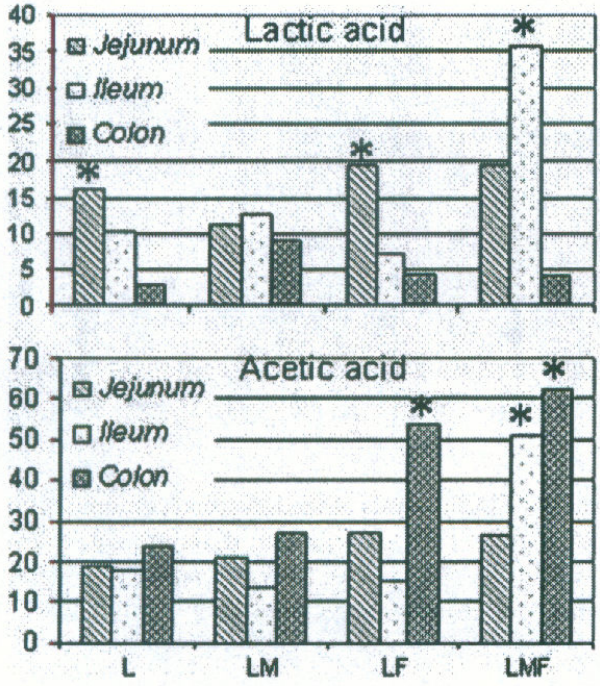

Figure 2. The concentration of organic acids (mmol/L) in the intestinal contents of 7-day-old conventional pigs. L, *Lact. plantarum* ($n = 8$); LM, *Lact. plantarum* + Maldex 150 ($n = 8$); LF, *Lact. plantarum* + Raftifeed IPX ($n = 8$); LMF, *Lact. plantarum* + Maldex 150 + Raftifeed IPX ($n = 9$)

plantarum and *Lact. plantarum* + Raftifeed IPX animals as well as in the ileal contents of *Lact. plantarum* + Maldex 150 + Raftifeed IPX animals. In the *Lact. plantarum* + Maldex 150 group, lactic acid concentrations were even in all gut segments. In the *Lact. plantarum* + Raftifeed IPX and *Lact. plantarum* + Maldex 150 + Raftifeed IPX groups, acetic acid concentrations reached maximum values in the contents of the colon ($p < 0.05$). No significant differences were seen within groups *Lact. plantarum* and *Lact. plantarum* + Maldex 150.

Chromatograms of samples from intestinal tract of piglets to whose feed Maldex 150 and Raftifeed IPX were added are shown in Figure 3. In all analysed samples of the gut from *Lact. plantarum* + Raftifeed IPX animals, individual components of Raftifeed IPX were detected visually on chromatograms in parts of the jejunum and ileum, respectively (mainly in the jejunum and partially in the ileum). The presence of individual unaltered components in the colon was not confirmed. In the colon, the presence of residual monosaccharide was confirmed. The individual components of maltodextrin were not confirmed in an unaltered state in any part of the intestinal tract (jejunum, ileum and colon) of *Lact. plantarum* + Maldex 150 animals. On the chromatograms, the spots can only be seen at the position of the monosaccharide. From their R_F values, the spots correspond to glucose.

DISCUSSION

Maltodextrin is defined as a non-sweet, nutritive saccharide polymer that consists of D-glucose units linked primarily by $\alpha(1\rightarrow4)$ glycosidic bonds and has a dextrose equivalent

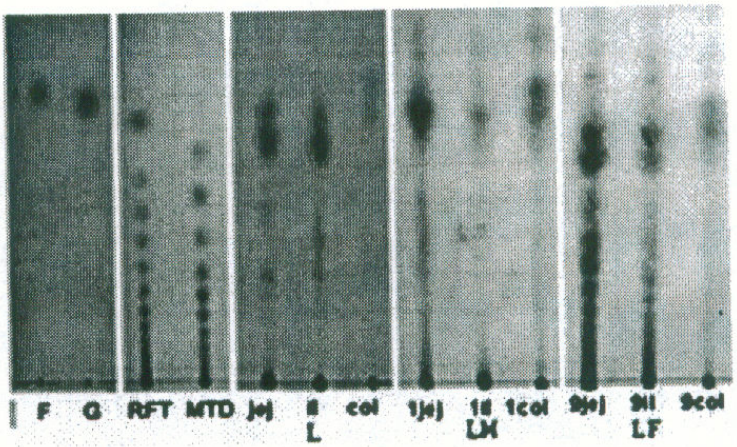

Figure 3. Chromatogram of the TLC analysis of the intestinal contents in pigs: F, fructose; G, glucose; RFT, Raftifeed IPX; MTD, Maldex 150; jej, jejunum; il, ileum; col, colon. L, *Lact. plantarum* ($n = 8$); LM, *Lact. plantarum* + Maldex 150 ($n = 8$); LF, *Lact. plantarum* + Raftifeed IPX ($n = 8$); LMF, *Lact. plantarum* + Maldex 150 + Raftifeed IPX ($n = 9$)

of less than 20. Maltodextrins are hydrolysed and absorbed in the small intestine so that it does not reach the colon intact. Maltodextrins are hydrolysed by pancreatic α-amylase to lower saccharides and α-limit-dextrins. Several membrane enzymes intrinsically bound to the epithelial cells of the intestinal mucosa, such as maltase, saccharase and α-dextrinase, can further hydrolyse these linear and branched saccharides to glucose (Marchal *et al.*, 1999). The results of TLC analysis of the gut contents of pigs receiving maltodextrin confirmed that the processes of hydrolysis and absorption of the latter take place in the small intestine, since no fractions were detected in the investigated gut segments. Nakakuki (1993) reported in his review that the consumption of maltotetraose-rich corn syrup had been demonstrated to reduce the levels of intestinal putrefactive bacteria such as *Clostridium perfringens* and members of the family *Enterobacteriaceae* in human trials. Bomba and colleagues (1999) investigated the influence of preventive administration of *Lactobacillus casei* subsp. *casei* and maltodextrin KMS X-70 on the adhesion of *E. coli* O8: K88 in the gastrointestinal tract of gnotobiotic piglets. *Lact. casei* subsp. *casei* administered together with maltodextrin decreased the *E. coli* counts on the jejunal mucosa of gnotobiotic piglets by 1 log in comparison to the *Lact. casei* subsp. *casei* group. The inhibitory effects of maltodextrin upon the counts of *E. coli* O8: K88 adhering to the jejunal mucosa of conventional pigs were confirmed in our experiments, since *E. coli* counts were decreased by 1.5 log in the group receiving Maldex 150 and *Lact. plantarum* when compared to animals receiving only *Lact. plantarum*. However, the decrease of *E. coli* counts in the *Lact. plantarum* + Maldex 150 group did not correlate with the increased production of organic acids in the jejunal content; this fact suggests that maltodextrin had no specific effect on lactobacilli. Our results revealed that in group *Lact. plantarum* + Maldex 150 administration of maltodextrin did not influence the total counts of lactobacilli in the gut content. The mechanism of this inhibitory action does not seem to consist in the potentiation of lactobacillar growth or acid production. In contrast, the inhibitory effect of *Lact. plantarum* and Raftifeed IPX upon *E. coli* counts in the colon may have been caused by increased acetic acid production due to the

induction of growth of the investigated strain or other beneficial intestinal bacteria. We found nonsignificantly higher counts of total lactobacilli in the contents of the jejunum, ileum and colon in groups *Lact. plantarum* + Maldex 150 + Raftifeed IPX and *Lact. plantarum* + Raftifeed IPX. TLC analysis of the gut contents of pigs receiving Raftifeed IPX confirmed total hydrolysis and absorption of the latter in the colon (presence of residual monosaccharide). The amount of fructooligosaccharides decreased from the jejunum to the colon and led to their fermentation. This is because the fructooligosaccharides are neither hydrolysed by the endogenous digestive enzymes nor absorbed by the host. They act as substrates for *Bifidobacterium*, *Lactobacillus* and *Streptococcaceae* (Simon and Jadamus, 2002), and after ingestion they stimulate the growth and metabolic activity of beneficial bacteria, which results in a decreased intestinal pH (production of organic acids or short-chain fatty acids), which in turn results in reduced concentrations of pathogens or potential pathogens (Gibson and Roberfroid, 1995; Zimmerman *et al.*, 2001; Gibson and Fuller, 2002). Similarly, a synergistic effect of the combination of *Lact. paracasei* and fructooligosaccharides (Raftilose) upon faecal microflora was observed in our previous experiments that used weaned pigs (Nemcová *et al.*, 1999). This effect was demonstrated by increased total counts of anaerobes, aerobes, lactobacilli and bifidobacteria, as well as by decreased counts of *Clostridium*, *Enterobacteriaceae* and *E. coli*.

In conclusion, the combination of *Lactobacillus plantarum*, Maldex 150 and Raftifeed IPX seems to have had the most pronounced inhibitory effect upon *E. coli* O8:K88 cells adhering to the jejunal and colonic mucosa. In our opinion, the experimental combination may be used to potentiate probiotic action in the intestine; however, the hypothesis requires further research.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Slovak Scientific Agency VEGA no.1/1337/04, and projects from the Research and Development Support Agency APVV-20-062505 and MVTS 047 CZE. The authors gratefully acknowledge the technical assistance of Jana Kakalejčíková and Ol'ga Hanzelová.

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(Accepted: 8 May 2006; Published online: 13 February 2007)