Effect of polydextrose on intestinal microbes and immune functions in pigs

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Dietary fibre has been proposed to decrease risk for colon cancer by altering the composition of intestinal microbes or their activity. In the present study, the changes in intestinal microbiota and its activity, and immunological characteristics, such as cyclo-oxygenase (COX)-2 gene expression in mucosa, in pigs fed with a high-energy-density diet, with and without supplementation of a soluble fibre (polydextrose; PDX) (30 g/d) were assessed in different intestinal compartments. PDX was gradually fermented throughout the intestine, and was still present in the distal colon. Irrespective of the diet throughout the intestine, of the four microbial groups determined by fluorescent in situ hybridisation, lactobacilli were found to be dominating, followed by clostridia and Bacteroides. Bifidobacteria represented a minority of the total intestinal microbiota. The numbers of bacteria increased approximately ten-fold from the distal small intestine to the distal colon. Concomitantly, also concentrations of SCFA and biogenic amines increased in the large intestine. In contrast, concentrations of luminal IgA decreased distally but the expression of mucosal COX-2 had a tendency to increase in the mucosa towards the distal colon. Addition of PDX to the diet significantly changed the fermentation endproducts, especially in the distal colon, whereas effects on bacterial composition were rather minor. There was a reduction in concentrations of SCFA and tryptamine, and an increase in concentrations of spermidine in the colon upon PDX supplementation. Furthermore, PDX tended to decrease the expression of mucosal COX-2, therefore possibly reducing the risk of developing colon cancer-promoting conditions in the distal intestine.

Intestinal bacteria: Immune responses: Polydextrose

The role of the gastrointestinal (GI) microbiota in health and disease has been widely studied in human subjects and animals. The GI microbiota is a highly diverse ecosystem established at birth, and its complexity is dependent on several factors, including the host’s genotype and physiology, the availability of nutrients from the diet and competitive mechanisms amongst the bacteria themselves (Savage, 1977). The composition of the GI microbiota and its activities directly influence the host’s health and may bring about beneficial effects or, on the contrary, pathogenic outcomes for the host (Gibson & Roberfroid, 1995; Salminen et al. 1998).

The main beneficial effects of an optimal microflora include resistance to colonisation by pathogens, for example, through adhesion mechanisms on the GI mucosa, competition for nutrients, the production of SCFA as endproducts of the fermentation of carbohydrates and proteins in the gut, and the production of antimicrobial compounds (for example, bacteriocins). In addition, the GI microbiota is important for mucosal integrity (Delzenne & Williams, 2002) and the development and regulation of intestinal immune responses, including oral tolerance towards food-borne antigen structures (Guaner & Malagelada, 2003; Mazmanian et al. 2005). The impact of the microbiota on chronic mucosal inflammation is rather unknown, but protective, anti-cancerous effects by probiotics and prebiotics via modulation of mucosal cyclooxygenase (COX) expression in vitro have been suggested (MäkivuoKKo et al. 2005; Nurmi et al. 2005). Harmful activities of the GI microbiota include the production of potentially toxic and carcinogenic compounds deriving from proteolytic metabolic processes (for example, ammonia, phenols), the translocation of opportunistic pathogens across the mucosal barrier to the mesenteric lymph nodes and other extra-intestinal sites (Cummings & Macfarlane, 1991; Gibson & Roberfroid, 1995) and GI infections caused by ingested pathogens leading to diarrhoea. Therefore, the modulation of the GI microbiota through the diet may be advantageous for the host.

As in man, the large intestine of pigs is the anatomical region most heavily and diversely populated by bacteria, with over 400 different bacterial species and in concentrations ranging from 1010 to 1012 microbial cells per g intestinal contents (Ewing & Cole, 1994; Berg, 1996). Furthermore, nutritional studies have shown that the swine is generally

Abbreviations: COX, cyclo-oxygenase; FISH, fluorescent in situ hybridisation; G + C, guanine + cytosine; GI, gastrointestinal; PDX, polydextrose.

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considered as an excellent animal model of man’s GI tract (Miller & Ullrey, 1987; Moughan et al. 1994). Several dietary components, such as fibre and prebiotics, can influence the intestinal functions and the digestive processes (digesta movement, digesta volume and digesta transit time) and therefore beneficially impact on the host’s health (Roberfroid, 1993; Freire et al. 2003). Furthermore, an increase in dietary fibre intake appears to decrease the risk of colon cancer relative to the amount of consumed fibre (Bingham, 2006). The mechanisms are largely unknown. However, the role of COX, especially COX-2 in several different cancer types, such as colon, gastric, skin and lung cancer, has been documented (Muller-Decker et al. 1999; Prescott & Fitzpatrick, 2000; Arbabi et al. 2001). Especially, the risk of colon cancer can be decreased or development even reversed by the use of COX-2-specific inhibitors (Krause & DuBois, 2001).

Polydextrose (PDX) is a randomly polymerised glucose oligosaccharide with an average degree of polymerisation of 12, which is used as a multi-purpose commercially available food ingredient. It is commonly used as a replacement for sugar, starch and fat in commercial food products, and has been previously shown to exhibit prebiotic properties and beneficial effects on mucosal activity (Jie et al. 2000; Peuranen et al. 2004; Probert et al. 2004; Mäkivuokko et al. 2005). In the present study, the fermentation of PDX was assessed in the small intestine, caecum and different parts of the colon in a porcine model. In addition, the microbial and immunological environment in the GI tract of pigs was further analysed by studying the composition and activity (SCFA and biomarkers of proteolytic activity) of the microbiota and immune functions, such as IgA secretion and mucosal COX gene expression.

Materials and methods

Animals and trial groups

The ethical approval for the trial was obtained from the Animal Care and Use Regional Ethical Committee of MTT (Agrifood Research Finland, Jokioinen, Finland). A total of twenty healthy pigs (eight females and twelve castrated males) were used in the trial. Of these, seven were Finnish Landrace, two Finnish Yorkshire, one cross of these breeds and ten were crosses of Landrace × Yorkshire sows and Duroc × Landrace boars. Their initial weight was 28 (± 1·5) kg and age 67 (± 9·5) d. The pigs were randomly allocated to two treatment groups which were balanced for sex, breed and litter origin. The pigs were housed individually in pens of 1·0 × 1·5 m with a slotted dunging area of 1·0 × 1·0 m. The acclimatisation period lasted 7 d and the experimental period 21 d. The purpose of the acclimatisation period was to familiarise the pigs with their experimental diets. Housing and environment of the pigs was kept similar during both periods.

Diet

The experimental treatments were (1) control (basal high-energy-density pig diet; no added PDX) and (2) basal diet supplemented with 30 g PDX/pig per d (Litesse® Ultra™; Danisco Sweeteners, Redhill, Surrey, UK). In the basal diet, 19·9 % of gross energy came from protein, 29·7 % from fat and 50·4 % from carbohydrates (Table 1). The nutrient composition of the diet ingredients was adopted from Finnish food composition tables (Rastas et al. 1993).

The basal diet was supplemented with minerals, vitamins and amino acids to fulfill the Finnish nutrient requirements of growing pigs (Tuori et al. 1995). Protein in the diet was mainly of animal origin and wheat flour and potato flakes were used as the main carbohydrate source to restrict the intake of dietary fibre. The fibre intake of pigs was 1·8 g/MJ, which approximates to 60 % of human Finnish fibre intake recommendations (3·0 g/MJ). The total dietary fibre content was 43·4 g/kg food; 44–62 g dietary fibre/d (2·2 g/MJ) per pig. Lower fibre concentrations would have increased the risk of stomach ulceration (very low viscosity allows the free flow of acidic secretions to flush the walls of the stomach), and would therefore have been detrimental for the health of the pigs. Health status of pigs was assessed twice daily by clinical monitoring, and no signs of illness were detected during the 4-week trial. The average daily weight gain of the pigs was 83·4 (± 72·7) g.

During the acclimatisation and experimental periods, the pigs were fed twice daily (07.00 and 15.30 hours) according to an age-based, restricted scale (Tuori et al. 1995). Their daily energy intake was 19·6–27·3 MJ. The feed intake of the pigs averaged 1378 g/d (32 g/kg live weight) and no

<table>
<thead>
<tr>
<th>Table 1. Composition of the high-energy-density basal diet</th>
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<tbody>
<tr>
<td>Components (g/kg)</td>
</tr>
<tr>
<td>Wheat flour*</td>
</tr>
<tr>
<td>Potato flakes</td>
</tr>
<tr>
<td>Soy protein flour†</td>
</tr>
<tr>
<td>Skimmed milk powder</td>
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<tr>
<td>Whey protein powder</td>
</tr>
<tr>
<td>Fish meal</td>
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<tr>
<td>Sugar</td>
</tr>
<tr>
<td>Butter</td>
</tr>
<tr>
<td>Rapeseed oil</td>
</tr>
<tr>
<td>L-Lysine HCl</td>
</tr>
<tr>
<td>DL-Methionine</td>
</tr>
<tr>
<td>L-Threonine</td>
</tr>
<tr>
<td>Limestone</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
</tr>
<tr>
<td>Mineral–vitamin premix‡</td>
</tr>
</tbody>
</table>

Calculated composition (g/kg)

| Gross energy, calculated (MJ/kg) | 17·0 |
| Crude protein, analysed | 219 |
| Crude fat, analysed | 133 |
| Dietary fibre, analysed | 43·4 |
| Ca, calculated | 10·4 |
| P, calculated | 8·3 |

* Standard baking flour milled from wheat endosperm (Raisio Ltd, Raisio, Finland).
† Ground non-GMO soy protein product HP 310 for feed application purposes (Hamlet Protein A/S, Horsens, Denmark).
‡ Vitamin–mineral mixture provided (per kg diet): P, 1·1 g; Ca, 3·1 g; Mg, 0·7 g; Fe, 143 mg; Cu, 31 mg; Se, 0·4 mg; Zn, 125 mg; Mn, 32 mg; I, 0·3 mg; NaCl, 4·5 g; vitamin A, 2·1 mg; vitamin D, 17·9 µg; vitamin E, 69 mg; vitamin K, 2·6 mg; vitamin B₁, 2·7 mg; vitamin B₆, 6·6 mg; vitamin B₁₂, 3·8 mg; vitamin B₉, 0·03 mg; biotin, 0·28 mg; pantothenic acid, 19·4 mg; niacin, 27·4 mg; folic acid, 4·6 mg.
refusals were left. The aim was to maintain normal growth of
the animals, however, with slightly restricted feed intake
(80–85 % ad libitum feeding), ensuring 100 % consumption
of PDX. In group 2, PDX was mixed in the basal control
diet during the experimental period: 15 g/d in the morning
and 15 g/d in the afternoon per pig.

Feed analyses
The N content of the diet was determined by a Dumas method
(method 968.06) using a Leco FP 428 nitrogen analyzer (Leco
Corp., St Joseph, MI, USA). The crude protein content was
calculated by multiplying the percentage of N by 6.25. Diethyl
ether extract was analysed after hydrolysis with 3 M-HCl
(method 920.39) (Anonymous, 1971). The total dietary fibre
was analysed according to AOAC 45-4.07/NMKL 129.

Sample collection
The pigs were killed with a stun gun and exsanguinations were
performed immediately by cutting the jugular veins, alternating
the pigs from control and PDX groups. Their live
weight at slaughter was 51.1 (sd 2.67) kg. During the blood-
letting, a blood sample was collected into 50 ml plastic tubes
and blood cells and plasma were immediately separated by a
short centrifugation (12000 g; 1 min). The small intestine,
caecum and colon of each pig were dissected, and the last
4 m covering the distal part of the small intestine was isolated.
At that point the intestines were tied with cable binders to
avoid loss of digesta. After binding, approximately 2 cm
long intestinal tissue pieces were excised from the point,
which was 4 m from the ileo-caecal junction, and mucus
was gently scraped from the tissue with a scalpel and sub-
merged in RNAlater (see below in the section Determination
of mucosal cyclo-oxygenase gene expression) (Qiagen,
Hilden, Germany), kept first during the sampling at room tem-
perature, and then stored at −20°C. In addition, a mucosal
tissue sample was obtained from the tip of the caecum (after
emptying the contents) and three mucosal tissue samples
were obtained from the colon at proximal, middle and distal
regions. All digesta from the proximal small intestine
(approximately 20 m), including the last 4 m, were collected.
The contents of the caecum and the large intestine, which
was divided into three equally long segments, were similarly
collected. Digesta from the distal small intestine, caecum
and the three caecal segments were divided into subsamples
for the determination of DM content, and IgA and chemical
and microbial analysis. These subsamples were kept on ice
during the sampling and subsequently stored at −20°C.

Determination of polydextrose concentrations
The concentration of native PDX was measured using an
HPLC method designed to identify the presence of native
PDX in foods as described previously (Craig et al. 2000).

Determination of dry matter contents and short-chain fatty
acid concentrations
The DM content of digesta was determined by weighing it
before and after drying at 105°C for 24 h. The SCFA in digesta
or plasma were analysed as follows: 1 ml of an internal stan-
dard (20 mM-pivalic acid) and 5 ml water were added to 1 g of
the sample. After thorough mixing, the sample was centri-
fuged at 5000 g for 5 min. Following centrifugation 0.25 ml
of saturated oxalic acid solution was added to 0.5 ml of the
supernatant fraction and the mixture was incubated at 4°C
for 60 min, and then centrifuged at 16000 g for 5 min. The
supernatant fraction or blood plasma samples were analysed
by GC essentially as described previously (Holben et al.
2002). The concentrations of acetic acid, propionic acid, buty-
ic acid, isobutyric acid, valeric acid, isovaleric acid,
2-methylbutyric acid and lactic acid were determined. Bio-
genic amines were determined from caecal digesta samples
according to Saarinen (2002). The results are expressed as
mmol SCFA/ml plasma, μmol SCFA/g digesta (wet weight),
mmol biogenic amine/g digesta (wet weight) and μmol
NH₃/g digesta (wet weight).

Determination of intestinal immunoglobulin A concentrations
IgA was determined from individual digesta samples treated
with an equal volume of 1.0 % bovine serum albumin solution
in 50 mM-tri(hydroxymethyl)-aminomethane (pH 7.5), 0.15 M-
NaCl for 60 min at room temperature. The samples were then
briefly centrifuged at 50000 g and the supernatant fractions
were used for IgA measurement. IgA was determined with
ELISA utilising specific antibodies and standard samples
obtained from Bethyl Laboratories, Inc. (Montgomery, TX,
USA) according to the manufacturer’s instructions. The results
were expressed as μg IgA/g digesta (wet weight).

Determination of mucosal cyclo-oxygenase gene expression
For the quantitative determination of COX-1 and -2
expression levels, tissue specimens were rinsed briefly in ster-
ile 0.9 % (w/v) NaCl solution and approximately 35 mg of the
epithelial cells were scraped off using a sterile scalpel. Total
RNA was stabilised immediately with the RNAlater reagent
(Qiagen, Hilden, Germany) according to the instructions pro-
vided by the manufacturer. Total RNA was extracted from the
samples using the RNeasy Mini Kit (Qiagen) and the contami-
nating genomic DNA was digested during RNA extraction
using the same manufacturer’s RNase-free DNase (Qiagen)
according to instructions provided by the manufacturer.
Then 2 μg RNA was reverse-transcribed using the High
Capacity cDNA Archive Kit (Applied Biosystems, Foster
City, CA, USA) according to the instructions provided by
the manufacturer. Absolute quantitative TaqMan PCR assays
were set up for primers (Applied Biosystems) detecting
specifically porcine COX-1 and COX-2, the sequences of
which are shown in Table 2. All assays were run on an ABI
Prism 7000 Sequence Detection System (Applied Biosystems)
using the instrument’s default settings for thermal cycling and
fluorescence measurements. Standard curves showing the
inverse log-linear relationship between initial template copy
number and the PCR cycle at which fluorescence intensity
crosses a background threshold value (the threshold cycle)
were prepared using spectrophotometrically quantified syn-
thetic oligonucleotides representing the antisense cDNA
sequence of each target transcript (data not shown). Results
are expressed as absolute transcript copy numbers/100 ng
total cellular RNA in oligonucleotide equivalents. When the effect of the PDX-supplemented diet on COX-gene expression was investigated, a relative percentage change for each part of the intestine was calculated by using the following formula:

% Change in COX – gene expression

= ((absolute copy number in PDX piglet

– mean absolute copy number in control group)/

mean absolute copy number in control group) × 100.

**Determination of microbial numbers by flow cytometry**

In order to determine the total number of microbes in caecal digesta samples, two methods were utilised: a fluorescent in situ hybridisation (FISH) method (explained later) and a flow cytometric method. The cells were separated from the collected digesta by differential centrifugation (Apajalahi et al. 1998). For counting, a sample of separated bacterial cells from each digesta sample was appropriately diluted and the cells were stained with a fluorescent, nucleic acid-binding dye (Syto 24; Molecular Probes, Eugene, OR, USA) (Apajalahi et al. 2002). Microbial numbers were determined by flow cytometry as previously described (Apajalahi et al. 2002). The results were reported as cells/g digesta (wet weight) and used for converting the relative proportions of bacteria in the percentage guanine + cytosine (G + C) profiling to numbers of bacteria.

**Microbial percentage guanine + cytosine profiling**

The %G + C profiling was performed as described previously (Apajalahi et al. 2001). In short, bacteria were separated from digesta samples by differential centrifugation after which the bacterial DNA was recovered from the cells by a combination of physical, chemical and enzymic lyses. DNA recovered from the total microbial community was then profiled based on the G + C content of the chromosomal DNA in the individual bacterial members of the community. Chromosomal DNA molecules (minimum of 300 μg DNA as pools of one to three samples) with different G + C contents were separated by CsCl density gradient centrifugation and the abundance of DNA monitored by pumping the solution through a UV flow cell. In order to analyse the effects of treatments on %G + C profiles, the profiles were divided into five increments, each covering 10% (covering an area between 26–75 %G + C). The proportion of microbes with %G + C belonging to a certain range of %G + C was calculated integrating the fractions from the %G + C profile.

**Microbial enumeration by fluorescent in situ hybridisation analysis**

For microbial enumeration using FISH, the homogenised digesta slurries in sterile ice-cold PBS (0·1 M-phosphate, pH 7·0) were centrifuged at 1500 g for 3 min to remove particulate matter and then fixed overnight in 4 % (w/v) paraformaldehyde. The bacterial cells were subsequently washed and re-suspended twice in sterile PBS and finally stored in PBS–ethanol at −20°C until hybridisation with appropriate molecular probes targeting 16S rRNA, as described by Ames et al. (1999). The probes used were Bif164 (Langendijk et al. 1995), Bac303 (Manz et al. 1996), CHis150 (Franks et al. 1998), Lab158 (Harmsen et al. 1999), specific for bifidobacteria, bacteroides, clostridia (Clostridium perfringens/histolyticum subgroup) and Lactobacillus/Enterococcus spp., respectively. The nucleic acid stain DAPI (4′,6-diamidino-2-phenylindole) was used for total bacterial counts. The DNA probes were tagged with the Cy3 fluorescence, enabling the examination of hybridised samples with fluorescence microscopy. Results are expressed as log10 cells/g digesta (wet weight).

**Statistical analyses**

To describe the intestinal environment in pigs, mean levels of measured characteristics (pH, NH3, SCFA, biogenic amines, FISH, COX-1, COX-2 and IgA) were compared between different compartments of the intestine. These mean levels of measured characteristics were compared in each part of the intestine between the control group and the group receiving PDX using two-sample t tests. %G + C profiling was also compared between the two groups using a t test. Furthermore, the statistical significances of the effect of PDX on COX-1 and COX-2 expression between different intestinal segments, and plasma concentrations of SCFA were calculated with a t test. P values < 0·05 were considered as significant.

**Results**

Twenty pigs were fed with a high-energy-density diet. The diet for half of the pigs was supplemented with 30 g PDX/d.

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**Table 2.** The sequences of oligonucleotide primers, probes and standards used in the present study

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence (5′ → 3′)</th>
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<tbody>
<tr>
<td>COX-1 forward primer</td>
<td>CAAATGGGCTTCGGCTTCA</td>
</tr>
<tr>
<td>COX-1 reverse primer</td>
<td>CCATAAATGCGCAGGTCTCA</td>
</tr>
<tr>
<td>COX-1 probe</td>
<td>FAM-CAAGGGTGGACGCCACGG-TAMRA</td>
</tr>
<tr>
<td>COX-1 standard</td>
<td>CCATAATGTGCGGAGGTTACCCCCGTGCGGCGCAAGCCTTGGTGAAAGCGAGCCACATCTTGT</td>
</tr>
<tr>
<td>COX-2 forward primer</td>
<td>CATTGATGGCAATGGACTGTA</td>
</tr>
<tr>
<td>COX-2 reverse primer</td>
<td>CTCCCCAAAGATGGCGACTTG</td>
</tr>
<tr>
<td>COX-2 probe</td>
<td>FAM-CTGCCCTTCGGTAGAACAGCCTCGCTAMRA</td>
</tr>
<tr>
<td>COX-2 standard</td>
<td>CTCCCCAAAGATGGCGACTTGCGGCGAGGTTTCTCCACAGAAGGCGAGGATAACACGCTCCATGGCATCAATG</td>
</tr>
</tbody>
</table>

COX, cyclo-oxygenase.

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Measurement of the PDX content of digesta DM taken from different parts of the intestine from the PDX group showed that concentrations of PDX decreased at an even rate, starting from approximately 53 mg/g DM in the distal small intestine and ending up at 35 mg/g DM in the distal colon, indicating that PDX was fermented more or less evenly throughout the intestine and was still present in the most distal part of the colon (Fig. 1).

Microbial metabolites

Several characteristics describing the microbial metabolites were measured from the different compartments of the intestine. The pH declined from the average of 6·5 measured in the distal small intestine to an average of 6·1–6·2 in the caecum. The pH increased gradually from the caecum to the most distal part of the colon (on average 6·5) even though the number of microbes remained high. The concentration of ammonia, on the other hand, increased steadily from the average of 10 μmol/g in the distal small intestine to over 40 μmol/g towards the distal colon. PDX supplementation had no effect on the pH or NH₃ concentrations (Fig. 2 (A)).

The differences of the total SCFA and biogenic amine concentrations for both treatments in different compartments of the intestine are depicted in Fig. 2 (B). The PDX addition to the diet had strongest influence on the colon fermentation pattern. Concentrations of the main SCFA (acetate, propionate, butyrate and valerate) were decreased in the colon in the group receiving PDX compared with the control group (Table 3). The changes became more evident towards the distal end of the colon where significant decreases could be observed with all SCFA including also all branched-chain fatty acids (iso-butyric acid, 2-methylbutyric acid and iso-valeric acid). Concomitant to lowered luminal SCFA, the plasma concentrations of the SCFA and lactic acid increased in the PDX-fed pigs. The mean plasma concentrations of acetic acid (11·5 (SE 1·70) mmol/l in control pigs and 12·4 (SE 1·32) mmol/l in PDX pigs), and especially lactic acid (7·5 (SE 1·32) and 10·8 (SE 2·12) mmol/l, respectively), appeared higher in pigs with the PDX-supplemented diet. Taken together, a tendency of a higher sum of the SCFA and lactic acid in the PDX fed group could be demonstrated (P=0·0758). Only trace amounts of butyrate and propionate could be detected (0·2 and 0·03 mmol/l, respectively), and no difference between the two diet groups could be detected. However, the plasma sum of branched SCFA (iso-butyric acid, 2-methylbutyric acid and iso-valeric acid) appeared lower in the PDX-supplemented group than in the control group (0·03 (SE 0·01) and 0·06 (SE 0·02) mmol/l, respectively; P=0·11; data not shown).
The concentrations of the most dominant biogenic amines, i.e. spermidine, putrescine, cadaverine, and methylamine, were low in the distal small intestine in control pigs (on average 83, 138, 188 nmol/g, and below detection level, respectively), increased approximately 3- to 9-fold in the beginning of the caecum, and then remained more or less constant throughout the large intestine or even decreased towards the end of the colon (see Table 3; data partially not shown).

The concentration of tryptamine was also below detection level in the distal small intestine in control pigs, and increased starting from the caecum. The concentration increased slowly towards the distal colon (on average 84 nmol/g), but did not reach concentrations as high as the four major biogenic amines (data not shown). Concentrations of biogenic amines were influenced by the PDX-supplemented diet. More specifically, concentrations of spermidine (and spermine) were increased in the distal colon in the group receiving PDX (Table 3). In contrast, the concentrations of tryptamine were decreased in the entire colon. As with SCFA concentrations, the PDX-induced changes on biogenic amine concentrations became more evident towards the distal end of the colon.

**Microbial community**

The composition of intestinal microbiota was characterised by G + C% profiling. A clear shift in the G + C% profiles from the proximal to the distal intestine was demonstrated irrespective to the diet (Fig. 3). However, the composition of the microbial community remained relatively unchanged by the PDX intervention as measured by %G + C profiling, though individual variation was high between animals (data not shown). After the profiles were divided into five increments,
each corresponding to an area of 10%G + C (range 26–75 %G + C), and multiplied with the corresponding total microbial numbers from fluorescence-activated cell-sorting analysis, a statistical comparison of the microbial abundances was possible. The abundance of microbes was increased by PDX treatment in the distal small intestine in the area of %G + C 26–35 ($P = 0.04$), 36–45 ($P = 0.0004$) and 66–75 ($P = 0.0025$), and in the caecum in the area of %G + C 26–35 ($P = 0.05$) and 36–45 ($P = 0.01$). In the proximal colon the abundance of microbes was decreased in four sequential increments (%G + C 36–75; $P = 0.02$, $P = 0.007$, $P = 0.004$ and $P = 0.03$, respectively). In the middle colon the abundance of microbes was increased in all five increments ($P = 0.05$, $P = 0.07$, $P = 0.05$, $P = 0.05$ and $P = 0.0002$, respectively) and in the distal colon in two increments: 36–45 ($P = 0.05$) and 56–65 ($P = 0.09$).

More detailed information on the intestinal distribution of bacterial population levels was obtained using FISH and oligonucleotide probes targeting important groups of the intestinal microbiota including *Bifidobacterium* species, *Bacteroides*, lactobacilli/enterococci and *Clostridium perfringens/histolyticum* subgroup. The FISH measurements of total microbial numbers from fluorescence-activated cell-sorting analysis, a statistical comparison of the microbial abundances was possible. The abundance of microbes was increased by PDX treatment in the distal small intestine in the area of %G + C 26–35 ($P = 0.04$), 36–45 ($P = 0.0004$) and 66–75 ($P = 0.0025$), and in the caecum in the area of %G + C 26–35 ($P = 0.05$) and 36–45 ($P = 0.01$). In the proximal colon the abundance of microbes was decreased in four sequential increments (%G + C 36–75; $P = 0.02$, $P = 0.007$, $P = 0.004$ and $P = 0.03$, respectively). In the middle colon the abundance of microbes was increased in all five increments ($P = 0.05$, $P = 0.07$, $P = 0.05$, $P = 0.05$ and $P = 0.0002$, respectively) and in the distal colon in two increments: 36–45 ($P = 0.05$) and 56–65 ($P = 0.09$).

Mucosal biomarkers

In addition to the changes in the luminal metabolites and microbes, biomarkers of immunological status in the intestine were monitored by measuring luminal IgA concentrations and microbial populations. The possibility of monitoring the microbial ecology of the porcine gut was found to be higher in the large intestine than in other parts (Jensen & Jorgensen, 1994). Also the *Lactobacillus/Enterococcus* population was found to be dominant in all of the animals, confirming previous results on the characterisation of porcine intestinal microbiota showing that this bacterial population is present at higher numbers in pigs compared with man (Barnes, 1986; Sghir et al. 2000; Mountzouris et al. 2006).

The %G + C profiling technique is a culture-independent method capable of depicting the total bacterial community within the GI tract in a single analysis. This method is capable of revealing large-scale shifts in the microbial community as a response to dietary changes (Apajalahti et al. 1998, 2002) without the need for conventional plating techniques. Differences in specific bacterial composition were observed for different regions of the gut irrespective of diet. The %G + C-profiling technique does not allow differentiation between different bacterial genera, but important information regarding the populations of major bacterial genera along the large intestine of pigs was generated by FISH, highlighting the possibility of monitoring the microbial ecology of the porcine gut. However, as a limitation to the present study, not all bacterial groups present in the porcine gut were enumerated with this selection of oligonucleotide probes. The existing FISH probes have been developed principally for human studies and may not give sufficient coverage for bacterial species relevant to porcine physiology. For instance, the porcine *Lactobacillus* microbiota differs from that found in man in both population size and species composition (Sghir et al. 2000). The highest concentration of the *Lactobacillus* population is present in the stomach and small intestine of pigs and it decreases towards the end of the pigs’ GI tract. Lactobacilli represent the major bacterial group in the swine GI microbiota, constituting 100% of the microflora in the stomach and between 90 and 100% of the microbiota in the duodenum of weaning piglets. In adult pigs lactobacilli constitute 30% of the microbiota in the stomach, while they are found at lower concentrations in the distal part of the GI tract (Sghir et al. 1998). On the contrary, lactobacilli are not found in the stomach of man and constitute less than 1% of the faecal microbiota (Mueller et al. 2006).

**In vitro** studies suggest that pig intestinal microbes ferment diverse types of oligosaccharides differently. Both the type...
The bacterial composition of digesta: numbers of *Bifidobacterium*, *Bacteroides*, lactobacilli/enterococci, *Clostridium perfringens/histolyticum*, and total number of cells measured by fluorescent in situ hybridisation (Mean values with their standard errors)

<table>
<thead>
<tr>
<th></th>
<th>Bifidobacterium</th>
<th>Bacteroides spp.</th>
<th>Lactobacilli</th>
<th>Clostridia</th>
<th>Total bacteria</th>
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<td>Mean SE</td>
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<tr>
<td>Distal small intestine Control</td>
<td>6·86 (\times 10^6) 2·46 (\times 10^6) &amp; 1·68 (\times 10^7) 8·00 (\times 10^6) &amp; 1·61 (\times 10^8) &amp; 8·06 (\times 10^7) &amp; 1·85 (\times 10^8) &amp; 5·94 (\times 10^6)</td>
<td>3·05 (\times 10^8) &amp; 5·49 (\times 10^8)</td>
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<tr>
<td>Caecum               Control</td>
<td>7·33 (\times 10^6) 2·91 (\times 10^6) &amp; 5·42 (\times 10^7) 2·97 (\times 10^6) &amp; 6·21 (\times 10^8) &amp; 1·69 (\times 10^8) &amp; 5·56 (\times 10^7) &amp; 3·20 (\times 10^7)</td>
<td>2·01 (\times 10^8) &amp; 5·06 (\times 10^7)</td>
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<tr>
<td>Proximal colon       Control</td>
<td>1·08 (\times 10^7) 3·47 (\times 10^6) &amp; 1·13 (\times 10^8) 7·54 (\times 10^7) &amp; 3·75 (\times 10^8) &amp; 1·20 (\times 10^8) &amp; 1·30 (\times 10^7) &amp; 7·63 (\times 10^6)</td>
<td>6·42 (\times 10^6) &amp; 8·29 (\times 10^6)</td>
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<tr>
<td>Middle colon         Control</td>
<td>1·98 (\times 10^7) 1·06 (\times 10^7) &amp; 1·63 (\times 10^8) 6·93 (\times 10^7) &amp; 2·56 (\times 10^8) &amp; 4·50 (\times 10^7) &amp; 5·03 (\times 10^7) &amp; 1·27 (\times 10^7)</td>
<td>6·37 (\times 10^6) &amp; 1·04 (\times 10^6)</td>
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<tr>
<td>Distal colon         Control</td>
<td>3·00 (\times 10^7) 8·48 (\times 10^6) &amp; 1·65 (\times 10^8) 4·54 (\times 10^7) &amp; 5·79 (\times 10^8) &amp; 1·16 (\times 10^8) &amp; 4·08 (\times 10^7) &amp; 8·37 (\times 10^6)</td>
<td>9·83 (\times 10^6) &amp; 1·25 (\times 10^6)</td>
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PDX, polydextrose.

Table 5. Luminal concentrations of immunoglobulin A, expression of cyclo-oxygenase (COX)-1 and COX-2 in tissue samples obtained from the intestinal compartments within control and polydextrose (PDX) groups, and effect of PDX on the COX expression (calculated as percentage difference compared with control)

(Means with their standard errors)

<table>
<thead>
<tr>
<th></th>
<th>COX-1 (mRNA copies/100 ng total RNA)</th>
<th>COX-2 (mRNA copies/100 ng total RNA)</th>
<th>Difference in COX gene expression (%)</th>
<th>IgA (µg/g digesta)</th>
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<tr>
<td></td>
<td>Control PDX</td>
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<tr>
<td>Distal small intestine Control</td>
<td>8952 789 8093 927 &amp; 13·425 3779 6635 1245 &amp; -9·6‡ 10·4 &amp; -50·6‡ 9·3 &amp; 379·50 59·03 355·93 62·33</td>
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<tr>
<td>Caecum               Control</td>
<td>25·212 2788 30·968 3381 &amp; 43·978 9591 34·227 7936 &amp; 22·6§ 15·2 &amp; 2·22 18·0 &amp; 38·25 13·54 48·59 19·41</td>
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<tr>
<td>Proximal colon       Control</td>
<td>37·965 6947 32·112 2656 &amp; 46·621 11·063 68·971 23·386 &amp; -15·4† 7·0 &amp; 47·9 50·2 &amp; 41·33 12·26 34·42 13·73</td>
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<tr>
<td>Middle colon         Control</td>
<td>27·919 2820 30·638 3205 &amp; 59·994 16·354 40·029 13·121 &amp; 9·7 11·5 &amp; -33·3 21·9 &amp; 36·66 19·14 13·89 4·88</td>
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<tr>
<td>Distal colon         Control</td>
<td>32·021 5795 27·690 6384 &amp; 125·782 46·470 44·425 13·659 &amp; -13·5 19·9 &amp; -64·7 10·9 &amp; 55·12 32·84 19·48 5·49</td>
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*P* value for the comparison with the distal small intestine (t-test).
†Mean value was significantly different from that of the caecum (*P* = 0·05) (t-test).
‡Mean value was significantly different from that of COX-1 (*P* = 0·01) (t-test).
§Mean value was significantly different from that of the COX-2 (*P* = 0·01).
||Mean value was significantly different from that of the proximal colon (*P* = 0·05).
and amount of SCFA produced vary, as well as the amount of gas produced (Smiricky-Tjarde, et al. 2003). Fermentation of PDX was observed to have significant effects on the microbial metabolic profiles but having rather small effects on the composition of the microbial community. In human clinical trials, and in the in vitro models, PDX has been shown to reduce putrefaction metabolites (branched-chain fatty acids) and increase SCFA production (Jie et al. 2000; Probert et al. 2004; Mäkivuokko et al. 2005; Mäkeläinen, In the press). However, more variable results have been obtained regarding the changes in composition of the microbial community (Jie et al. 2000; Probert et al. 2004; Mäkivuokko et al. 2005). Lack as well as presence of a bifidogenic effect has been shown both in vitro and in vivo.

Various factors could be considered as a reason for the lack of a prebiotic effect when certain oligosaccharides have been applied to swine diets. First, experimental animals are in good health and optimally reared using uniform balanced and highly nutritious diets in controlled and hygienic environments (Mikkelsen et al. 2003). Second, other non-digestible oligosaccharides or NSP in the diet could induce a dilution effect. Third, the intestine of pigs may harbour a high number of lactobacilli and bifidobacteria (Barnes, 1986; Gabert et al. 1995; Mathew et al. 1998; Franklin et al. 2002) that can reduce their response to prebiotic supplementation measured in the colon or in faeces. In human subjects, the bifidogenic effect of prebiotic oligosaccharides is inversely related to the volunteer’s initial counts (Van Loo et al. 1999). As with other single-stomached animals, pigs acquire a more stable GI microbiota only after weaning (Ewing & Cole, 1994; Adami & Cavazzoni, 1999). Perhaps the most appropriate times for demonstrating prebiotic effects in swine are stressful periods such as weaning. Stress is known to influence the GI microbiota and affect total lactobacilli populations (Mathew et al. 1998; Tannock, 2001; Franklin et al. 2002). Adult animals gradually adapted to the experimental diets were not under stressful conditions and might have had a more stable microbiota, showing less or no prebiotic effect.

In the present study, decreased concentrations of SCFA by the PDX treatment were measured. In vitro colon simulation experiments utilising complex microbiota (Probert et al. 2004; Mäkivuokko et al. 2005), all major SCFA (acetic acid, propionic acid and butyric acid) were increased upon PDX fermentation. Therefore, the decrease in the pig lumen may indicate increased uptake by epithelial cells rather than reduced production by microbes. In vitro fermentation of PDX was found to take place throughout the large intestine, explaining detection of beneficial effects even in the most distal colon. Increased spermidine, and a tendency of spermine, concentrations in the PDX group in the distal large intestine indicated improved epithelial cell growth (Heby, 1981). Furthermore, the principal reduction in production of branched-chain fatty acids as an indication of reduced putrefaction observed upon PDX supplementation in the distal large intestine is noteworthy, since the risk of developing colon cancer in the distal colon is especially high, and some of the putrefactive metabolites, such as indoles and phenols, have been shown to have carcinogenic effects (Smith & Macfarlane, 1997). By supplementing a low-fibre high-energy-density diet with such non-digestible carbohydrates that can persist until the distal colon and modify the microbial metabolism there towards a reduction in putrefaction may impact on the risk of developing colon cancer. Importantly, it was shown in the present study that PDX is fermented gradually throughout the colon and reaches also the most distal parts of the colon.

Prostaglandins derived from COX-2 are important in the healing of mucosal injury, in protecting against bacterial invasion, and in down regulating the mucosal immune system. However, increased COX-2 expression has been observed in colorectal carcinogenesis (Wendum et al. 2004; Wallace & Devchand, 2005). In the present paper we report for the first time that in healthy piglets fed with a high-energy-density diet the expression of COX-2 increased towards the distal end of the colon, although individual variation was quite high. Here, supplementation of the diet with PDX had a tendency to decrease the COX-2. As it is likely that in man expression of COX-2 varies from one individual to another, these data obtained from pigs may reflect the human situation in a reasonable way. A low-fibre diet may create an environment in the distal colon in sensitive individuals that favours cancer development. Increasing the weight of these preliminary findings, in a chemically induced colon cancer model, PDX has been shown to reduce numbers of aberrant crypt foci, especially in the distal colon (Ishizuka et al. 2003) and in an in vitro model, combining a colon fermentation simulation model with a Caco-2 cell model (Mäkivuokko et al. 2005), a dose-dependent reduction towards the distal colon in COX-2 expression was noted by PDX.

In a rat model an immune-stimulatory effect in the intestine by PDX has been previously suggested (Peuranen et al. 2004). In the present study no increase in IgA concentrations was detected. If stimulation is mediated by specific microbes, the difference may be explained by different microbiota in the two animal species, rat and pig.

In conclusion, the stable nature of the microbial community structure in pigs appears not to allow detection of minor effects induced by diet. Metabolic changes, on the other hand, are more readily detected and effects induced by PDX were evident and, furthermore, appeared similar to those retrieved from previous in vitro models and clinical studies. A gradual fermentation of PDX was found to take place throughout the large intestine, explaining detection of beneficial effects even in the most distal colon.

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References


