

## Combination of polydextrose and lactitol affects microbial ecosystem and immune responses in rat gastrointestinal tract

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The effects of various dietary fibres on gut health have been studied extensively but their combined effects are scarcely documented. In the present study the effects of 2% (w/w) polydextrose (PDX), 2% (w/w) disaccharide lactitol, or 2% (w/w) PDX+2% (w/w) lactitol on gut microflora, microbial metabolism and gut immune responses were investigated in rats. Both PDX and lactitol alone had an effect on many of the studied parameters, but their combination had stronger than additive effects in some parameters. The PDX+lactitol combination altered the microbial community structure as seen by a culture-independent method, percentage guanine+cytosine (%G+C) profiling, increasing the areas of %G+C 35–39 ( $P<0.0001$ ) and %G+C 45–49 ( $P=0.0002$ ), and decreasing %G+C 65–74 ( $P<0.0003$ ). These changes were also reflected in the microbial metabolism so that the production of biogenic amines and branched volatile fatty acids was significantly reduced, by 12 ( $P=0.03$ ) and 50% ( $P=0.002$ ), respectively, indicating a shift from putrefactive towards saccharolytic metabolism. PDX increased the secretion of IgA in the caecum ( $P=0.007$ ). Secretion of IgA increased even more, almost ten-fold, with the combination of PDX+lactitol ( $P<0.0001$ ) when compared with the control group. Lactitol increased the production of butyrate by caecal microbes by two- to three-fold when compared with the PDX or control group ( $P<0.0001$ ). Butyrate is a preferred energy source for mucosal cells; thus a boost in the availability of energy for immune cells may have still added to the synergistic effects of PDX and lactitol on immune cells. It is noteworthy that improvement in the IgA secretion occurred without signs of mucosal inflammation.

### Polydextrose: Lactitol: Microbial metabolism: Immunoglobulin A

Polydextrose (PDX) is a randomly bonded glucose polymer and, depending on its purity, contains residual sorbitol (6%) but no free glucose. It has a low energy value (4.2 kJ/g), and it appears to fit into the physiological definition of dietary fibre (for recent discussions on the definitions, see Hara *et al.* 2000; Craig *et al.* 2001). PDX is used as a bulking agent, and as a sugar or fat replacer in the food industry. Earlier studies have shown that PDX improves bowel movement and increases residual SCFA levels in human subjects (Jie *et al.* 2000). The laxative threshold of PDX in man is as high as 90 g/d (Pfizer, 1978).

Lactitol is a sugar alcohol made industrially by lactose hydrogenation. It is used as a low-energy sweetener (9.7 kJ/g) and is a suitable dietary component for diabetic patients (Natah *et al.* 1997). Being a non-absorbable sugar alcohol (Patil *et al.* 1987), it increases the water content of the intestine and reduces transit time (Lee & Storey, 1999; Soontornchai *et al.* 2003). Lactitol has been shown to reduce  $\text{NH}_3$  and amine levels in the digesta (Piva *et al.* 1996). The proposed mechanism for this is a reduced residence time of the intestinal contents, which reduces the time for  $\text{NH}_3$  generation and acidifies the intestinal lumen (Watanabe *et al.* 1995; Masini *et al.* 1999).

The effects of combinations of different C sources on colon function and its microbial metabolism are not extensively studied. The combination of 7% readily fermenting gum arabic and 7% non-fermenting cellulose changed the caecal fermentation pattern in rats in comparison with those at 14% concentrations alone (Topping *et al.* 1985). Combining 3% PDX with either pectin or cellulose increased the water-holding capacity of faeces and faecal weight in rats (Oku *et al.* 1991). Solomons & Rosenthal (1985), based on residual  $\text{H}_2$  in breath, found that PDX in combination with foods was more readily fermented than alone. Livesey *et al.* (1993) observed that PDX in combination with lactitol increased breath  $\text{H}_2$  production more than could be anticipated from their  $\text{H}_2$  production separately. Govers *et al.* (1999) studied the effect of slowly fermenting insoluble NSP on the fermentation of rapidly fermenting resistant starch in different intestinal sections of pigs. According to the authors, by increasing the bulk of digesta, NSP (wheat bran) speeded up the transit time of digesta, which then pushed resistant starch to be fermented into the distal colon producing SCFA.

In the present study we examined the effects of PDX (Litesse Ultra), lactitol and their combination on rats, concentrating on the gut microflora, microbial metabolites and

immunological responses. A percentage guanine+cytosine (%G+C) profiling method, independent of the culturability of microbes, was applied in the present study to examine diet-related changes in the gut microbial community (Apajalahti *et al.* 2001). In addition to changes in the microflora composition, we also measured indicators of microbial metabolism such as pH, SCFA and biogenic amines. Several indicators of the gut immune system were also included in the present study. Pro-inflammatory cytokines (IL-1 and TNF- $\alpha$ ) and a non-inflammatory cytokine (transforming growth factor (TGF)- $\beta$ ) produced by the intestinal immune and/or epithelial cells, and the crypt:villus measurement from histological samples reflect the immunological status of the gut mucosa (Olson *et al.* 1993; Letterio & Roberts, 1998). The measurement of IgA in the digesta characterises resistance against pathogens via neutralising antibodies that prevent adhesion on the epithelium, and tolerance against dietary components (Smith *et al.* 2000). A well-balanced regulation of immune responses in the intestinal environment has been proposed to be characterised by high IgA levels (for example, Kelsall & Strober, 1999).

## Materials and methods

### *Animals and experimental design*

Male Wistar rats (4 weeks old) were obtained from the Biocentre of the University of Helsinki and housed in a Scantainer cabinet with controlled temperature ( $27 \pm 2^\circ\text{C}$ ) and a 12 h light–12 h dark cycle. The animals had free access to food and water.

Forty-eight rats were divided into four feeding treatments; three animals per cage. The rats started to feed on the experimental diet on the day they arrived at the age of 4 weeks (weight 85.3 (SE 1.9) g). Before that, they were given rat feed in their nursery. The experiment lasted 3 weeks. The animals were weighed once weekly. The feeding groups were: control diet without non-digestible fibre (see later); PDX (2%, w/w); lactitol (2%, w/w); PDX (2%, w/w)+lactitol (2%, w/w).

At the end of the intervention period, the rats were anaesthetised with CO<sub>2</sub> and killed by cervical dislocation. The trial protocol was approved by the ethical committee of the University of Helsinki, Finland.

### *Diets*

A low-fibre diet (calculated fibre content 1%, w/w) was prepared for the rats (standard rat chow contains approximately 3.5% (w/w) crude fibre and 58% (w/w) N-free extract). The amounts of energy coming from different sources were calculated as 32, 31 and 36% for carbohydrates, protein and fat, respectively. The control diet contained (% w/w): peeled potatoes, 38.5; minced beef, 23.3; white wheat bread, 8.1; eggs, 20.4; butter, 1.6; sugar, 8.1. The ingredients were homogenised, mixed and baked in a steam oven at 200°C for 2–3 h. The mixture was allowed to cool to room temperature and the following ingredients were mixed in (% w/w): vitamin mixture (1324; Altromin International, Lage, Germany), 0.87;

cholesterol (Sigma, St Louis, MO, USA), 0.377; salt (NaCl), 2.0. The diets were stored at  $-20^\circ\text{C}$ .

The following feed analyses results were obtained with bomb calorimetry, inductively coupled plasma emission spectrometry and ion chromatography: heat value, 6.49 kJ/g; DM, 34.2%; ash, 0.91%; Na, 0.64 mg/g; Ca, 0.1 mg/g; K, 3.2 mg/g; Mg, 0.2 mg/g; Cl, 1.2 mg/g; phosphate, 2.5 mg/g. The total fat content in the diet was 4.8%. The fatty acid profile was analysed by GC. The basal diet had a total fatty acid content of 3.5%, a saturated fatty acid content of 1.7% and an unsaturated fatty acid content of 1.8%. The saturated:unsaturated fatty acid ratio was 0.9.

Polydextrose (Litesse Ultra, lot no. V68011; Danisco Sweeteners Ltd, Redhill, Surrey, UK) and lactitol (Xyrofin N125/22459; Redhill, Surrey, UK) were added in the basal diet at a 2% (w/w) concentration, which was calculated on the basis of human fibre intake recommendations (25–35 g/d) and the energy requirements of rats. Based on the energy value of the basal diet the feed consumption of a rat was approximated at 40 g/d. The fibre content of the diet was then designed to be at a moderate 2% level (20 g/kg diet). This was also the level of fibre that had previously been used in trials studying its degradation in rats (Djouzi *et al.* 1995). Oku *et al.* (1991) reported that 3% PDX did not cause diarrhoea in rats.

### *Sampling*

DM and IgA measurements were taken from individual ileal and caecal digesta samples (see later; p. 906). The rest of the caecal digesta was divided into pools (three animals per pool) for the analysis of SCFA, biogenic amines, total microbial counts and %G+C analysis. The samples were stored at  $-20^\circ\text{C}$  until analysis. pH was measured from the caecum at sampling with a ROSS combination pH electrode (model 82-63 PerHecT; Woburn, MA, USA). Intestinal tissue samples for histological and immunohistological analysis were obtained from the ileum and distal caecum. The sample was placed in a Tissue-Tek<sup>®</sup> OCT (Sakura Finetek, Zoeterwoude, The Netherlands), frozen in liquid N<sub>2</sub> and stored at  $-70^\circ\text{C}$ . The tissue samples were then cut with a cryostat microtome at 7  $\mu\text{m}$ , and the slices were placed on SuperFrost<sup>®</sup> microscope slides (Menzel-Gläser, Braunschweig, Germany), air-dried and stored at  $-70^\circ\text{C}$  until antibody staining (see later; p. 907).

### *Chemical analyses from digesta*

The DM content of digesta was determined by weighing before and after drying at 105°C for 2 h. The SCFA in digesta were analysed as follows: an internal standard (1 ml; 20 mM-pivalic acid) and 5 ml water were added to 1 g of the sample. After thorough mixing, the sample was centrifuged at 5000g for 5 min. Following centrifugation, 0.250 ml saturated oxalic acid solution was added to 0.500 ml of the supernatant fraction and the mixture was incubated at 4°C for 60 min, then centrifuged at 16 000g for 5 min. The supernatant fraction was analysed by GC as described previously (Holben *et al.* 2002).

The concentrations of acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, 2-methylbutyric acid and lactic acid were determined. Biogenic amines were determined from caecal digesta samples according to Saarinen (2002).

#### Microbial analyses

In order to determine the total number of microbes in caecal digesta samples, the cells were separated from the collected digesta by differential centrifugation (Apajalahti *et al.* 1998). For counting, a sample of separated bacterial cells from each caecal digesta pool (four per treatment, sixteen altogether) was appropriately diluted and the cells were stained with a fluorescent, nucleic acid-binding dye (Syto 24; Molecular Probes Inc, Eugene, USA) (Apajalahti *et al.* 2002). Microbial numbers were determined by flow cytometry as previously described (Apajalahti *et al.* 2002). The results were reported as cells/g wet caecal digesta.

The microbial %G+C profiling procedure was followed using the steps described in Apajalahti *et al.* (1998). In short, bacteria were separated from digesta samples by differential centrifugation. DNA was then recovered from the bacterial cells by a combination of physical, chemical and enzymic lyses. DNA recovered from the total microbial community was then profiled based on the guanine+cytosine content of the chromosomal DNA in the individual bacterial members of the community. Individual chromosomal DNA with different guanine+cytosine contents were separated by CsCl density gradient centrifugation and the abundance of DNA monitored by pumping the solution through a UV flow cell. The %G+C profiling technique is a culture-independent method capable of depicting the total bacterial community within the gastrointestinal 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. This means that changes in the entire microbial community are seen at one glance, even when of unknown identity. More resolution can be achieved when %G+C profiling is combined with, for example, 16S rDNA sequence analysis (Apajalahti *et al.* 2002).

#### Immunological analyses

Individual digesta samples were treated with equal volumes of 1.0% (w/v) bovine serum albumin solution in 50 mM-tris(hydroxymethyl)-aminomethane (pH 7.5)–0.15 M-NaCl for 60 min at room temperature. The samples were then briefly centrifuged at 50 000 g and the supernatant fractions were used for IgA measurement. Ig A 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 are expressed as µg Ig A/g digesta (wet weight).

Frozen sections of ileum and caecum were stained immunohistochemically to indicate the presence of IL-1- (Serotec Ltd, Oxford, UK), TGF-β- and TNF-α- (both

from R&D Systems, Minneapolis, MN, USA) positive cells in the mucosa. Antibodies were used at 1:100 dilutions. Goat anti-mouse antibodies (Southern Biotechnology Associates, Birmingham, AL, USA) were used as a secondary antibody (1:400 dilution). The cells were visualised by ABC Vectastain Elite kit PK-6100 system (Vector Laboratories, Inc., Burlingame, CA, USA). The enumeration of positively stained cells was done with image-based analysis (ImagePro 3.0; Media Cybernetics, Silver Spring, MD, USA) from photographed, microscopically randomly chosen sectors of tissue sections. Digesta particles, artefacts and connective tissue were removed with a photo editor before counting. The abundance of positive cells is expressed as a percentage of villus and crypt area of the mucosa.

#### Histomorphometrical measurements

The crypt:villus ratio of the ileum was determined from light micrographs by measuring the height of the crypts and villi. Areas where the plane of sections showed the ileal villi in their full length were selected for the measurements.

#### Statistical analysis

In order to analyse the effects of treatments on %G+C profiles, the %G+C profile was divided into twelve %G+C increments, each covering 5%. 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 (Apajalahti *et al.* 2001).

*Multiple linear regression.* Relationships between a single response (y variable) and scaled (from 0 to 1) x variables were analysed by multiple linear regression modelling (SYSTAT for Windows, version 5 edition; SPSS Inc., Chicago, IL, USA). The multiple linear regression model used for pooled data was:

$$y_i = C_{00} + C_{01} \times \text{Lac} + C_{02} \times \text{PDX} + C_{03} \times \text{Lac} + \text{PDX},$$

where Lac is (0,1), the variable that gets the value 1 for treatments where lactitol was amended (lactitol and lactitol+PDX); PDX is (0,1), the variable that gets the value 1 for treatments where PDX was amended (PDX and lactitol+PDX); Lac+PDX is (0,1), the variable that gets the value 1 for treatments where both lactitol and PDX were amended (lactitol+PDX);  $C_{00}$ ,  $C_{01}$ ,  $C_{02}$  and  $C_{03}$  are model parameters.

The multiple linear regression model essentially gives identical results to ANOVA.

The relative increase or decrease of certain treatments compared with the control treatment and the *P* value associated with that change are reported.

#### Results

The growth of rats during the 3-week period did not differ between the dietary groups (Table 1). The addition of PDX, lactitol or their combination significantly decreased the caecal pH ( $P < 0.0001$ ) (Table 1). PDX significantly

**Table 1.** Weight gain during 3 weeks, caecal pH, caecal dry matter and ileal dry matter (twelve rats per group)† (Mean values with their standard errors)

| Group...       | Control |      | PDX    |      | Lactitol |      | PDX+lactitol |      |
|----------------|---------|------|--------|------|----------|------|--------------|------|
|                | Mean    | SE   | Mean   | SE   | Mean     | SE   | Mean         | SE   |
| Growth (g)     | 134.2   | 3.1  | 129.2  | 3.6  | 131.9    | 3.3  | 124.7        | 3.2  |
| Caecum pH      | 6.39    | 0.05 | 5.70*  | 0.09 | 5.85*    | 0.06 | 5.81*        | 0.11 |
| Caecum DM‡ (%) | 19.41   | 0.25 | 22.14* | 0.37 | 20.21    | 0.39 | 20.23        | 0.26 |
| Ileum DM‡ (%)  | 19.93   | 0.21 | 21.52* | 0.27 | 20.43    | 0.16 | 20.43        | 0.51 |

PDX, polydextrose.

\*Mean value was statistically significantly different from that for the control ( $P < 0.01$ ).

†For details of diets and procedures, see p. 906.

‡DM content calculated from four pools.

increased the DM content of ileal ( $P = 0.002$ ) and caecal ( $P = 0.0001$ ) digesta (Table 1).

PDX alone and in combination with lactitol reduced the concentrations of total SCFA in the caecum. However, the effect was statistically significant only in the combination ( $P < 0.0001$ ; Table 2). The concentration of acetic acid was significantly reduced by PDX (23%;  $P < 0.0001$ ), lactitol (15%,  $P = 0.0005$ ), and their combination (38%,  $P = 0.0005$ ; Table 2). Lactitol increased the concentration of butyric acid by 187% ( $P < 0.0001$ ), while together with PDX the increase from control was 70% ( $P < 0.0001$ ). PDX ( $P < 0.0001$ ), lactitol ( $P < 0.0001$ ) and their combination ( $P = 0.0002$  to  $P = 0.002$ ) significantly reduced the concentrations of branched SCFA isobutyric acid, 2-methylbutyric acid and isovaleric acid (Table 2). Lactic acid concentration was significantly increased by the addition of lactitol (46%,  $P = 0.02$ ), whereas the addition of PDX in combination with lactitol significantly reduced it (39%,  $P = 0.0003$ ). The relative amount of acetic acid was significantly reduced by the addition of PDX, lactitol or their combination ( $P < 0.001$ ), while that of propionic acid increased significantly (Table 2). Lactitol alone or in combination with PDX significantly increased the relative amount of butyric acid ( $P < 0.001$ ). For lactic acid, the relative changes were small, yet significant by the addition of lactitol (increase;  $P < 0.05$ ) and combination of PDX and lactitol (decrease;  $P < 0.05$ ). The relative amount of branched SCFA was reduced by the addition of lactitol, PDX or their combination ( $P < 0.01$ ).

The total number of microbes in the caecum decreased significantly when PDX, lactitol or their combination were added in the diet; the reduction was 22% ( $P = 0.009$ ), 24% ( $P = 0.004$ ), and 13% ( $P = 0.006$ ), respectively (Table 3). All dietary treatments changed the structure of the caecal microbial community, but the combination of PDX and lactitol had a stronger effect on the microbial community than either of them alone. Both PDX and lactitol increased the abundance of bacteria within %G+C 40–44 by 27% ( $P = 0.01$ ) and 24% ( $P = 0.02$ ), respectively (Figs. 1 (a) and (b)). The PDX+lactitol diet resulted in a 32% increase in the %G+C 35–39 area ( $P < 0.0001$ ), and a 15% increase in the %G+C 45–49 area ( $P = 0.0002$ ). Also, the PDX+lactitol diet resulted in a 35–49% reduction in a wide area of %G+C 50–74 ( $P = 0.0003$  between area %G+C 65–74) in comparison with the control (Fig. 1 (c)). The PDX

diet resulted in a maximum of 22% reduction in the area between %G+C 50–69 ( $P = 0.0001$  at %G+C 55–59). The lactitol diet also gave a significant reduction in the area between %G+C 50–59 ( $P = 0.001$ ) (Figs. 1 (a) and (b)).

The concentrations of biogenic amines and their relative changes with different diets in the caecum are given in Table 4. The sum of biogenic amines reduced by 12% with the combination of PDX and lactitol ( $P = 0.03$ ; Table 4). The highest residual concentrations of individual biogenic amines were measured for spermidine and methylamine. Methylamine concentrations were decreased significantly by the addition of PDX, lactitol and their combination (Table 4). Lactitol alone significantly increased the concentration of spermidine but in combination with PDX it was significantly reduced. The concentration of putrescine was increased significantly by PDX, lactitol and their combination. Tyramine concentration was increased by PDX and by the PDX+lactitol combination but was reduced by lactitol (Table 4). Lactitol, PDX and their combination significantly increased the concentration of histamine in the caecal digesta (Table 4).

There were no significant differences in the crypt:villus ratio of rat small intestine with the different diets indicating a lack of irritating stimulus on the epithelium. The crypt:villus ratios were: control, 0.53 (SE 0.04); PDX group, 0.53 (SE 0.05); lactitol group, 0.50 (SE 0.06); PDX+lactitol group, 0.55 (SE 0.04). However, PDX, lactitol and their combination significantly ( $P < 0.01$ ) reduced the villus height ( $\mu\text{m}$ ): control, 135.83 (SE 10.61); PDX group, 98.15 (SE 5.19); lactitol group, 105.00 (SE 7.62); PDX+lactitol group, 96.48 (SE 8.83). Similarly, the crypt depth reduced significantly ( $P < 0.05$ ) in these groups ( $\mu\text{m}$ ): control, 70.63 (SE 7.24); PDX group, 52.41 (SE 4.86); lactitol group, 50.19 (SE 4.15); PDX+lactitol group, 52.59 (SE 4.98).

Diet had no effect on the Ig A concentrations in the rat ileum. However, the concentration of Ig A in the caecum increased several-fold (345%) by PDX ( $P = 0.007$ ), and by the combination PDX+lactitol (996%,  $P < 0.0001$ ; Fig. 2). PDX alone increased the ileal IL-1-positive cells significantly ( $P = 0.007$ ) but, when combined with lactitol, no effects were seen (Table 5). The test diets had no effect on the IL-1-positive or TNF- $\alpha$ -positive cells in the caecum when compared with the control diet. Lactitol increased the area of TGF- $\beta$ -positive cells in the caecum

**Table 2.** Residual concentrations (mm) of short-chain fatty acids and lactic acid in the caecum of rats (twelve rats per group)† (Mean values with their standard errors)

| Group...             | Control |      |      | PDX    |                       |       | Lactitol |      |       | PDX+lactitol |                       |       |
|----------------------|---------|------|------|--------|-----------------------|-------|----------|------|-------|--------------|-----------------------|-------|
|                      | Mean    | SE   | %    | Mean   | SE                    | %     | Mean     | SE   | %     | Mean         | SE                    | %     |
| Acetic acid          | 73.83   | 5.18 | 75.7 | 56.50* | 3.35                  | 70.9* | 62.50*   | 1.72 | 60.8* | 46.16*       | 3.30                  | 63.2* |
| Propionic acid       | 10.50   | 0.83 | 10.8 | 12.00  | 0.66                  | 15.1* | 11.75    | 1.11 | 11.4* | 10.92*       | 0.83                  | 15.0* |
| Butyric acid         | 8.33    | 0.99 | 8.5  | 7.58   | 0.92                  | 9.5   | 22.83*   | 1.35 | 22.2* | 13.50*       | 0.87                  | 18.5* |
| Lactic acid          | 3.92    | 0.58 | 4.0  | 3.27   | 0.54                  | 4.1   | 5.25*    | 0.50 | 5.1   | 2.18*        | 0.23                  | 3.0*  |
| Branched SCFA        |         |      |      |        |                       |       |          |      |       |              |                       |       |
| Isobutyric acid      | 0.50    | 0.04 | 0.5  | 0.23*  | 0.03                  | 0.3*  | 0.27*    | 0.03 | 0.3*  | 0.15*        | 0.02                  | 0.2*  |
| 2-methylbutyric acid | 0.21    | 0.01 | 0.2  | 0.10*  | 5 × 10 <sup>-10</sup> | 0.1*  | 0.12*    | 0.01 | 0.1*  | 0.10*        | 5 × 10 <sup>-10</sup> | 0.1*  |
| Isovaleric acid      | 0.23    | 0.02 | 0.2  | 0.10*  | 5 × 10 <sup>-10</sup> | 0.1*  | 0.12*    | 0.01 | 0.1*  | 0.10*        | 5 × 10 <sup>-10</sup> | 0.1*  |
| Sum of branched      | 0.94    |      |      | 0.43*  |                       |       | 0.50*    |      |       | 0.36*        |                       |       |
| Sum of all           | 97.5    |      |      | 79.7   |                       |       | 102.8    |      |       | 73.0*        |                       |       |

PDX, polydextrose.

\*Mean value was statistically significantly different from that for the control ( $P < 0.05$ ).

† For details of diets and procedures, see p. 906.

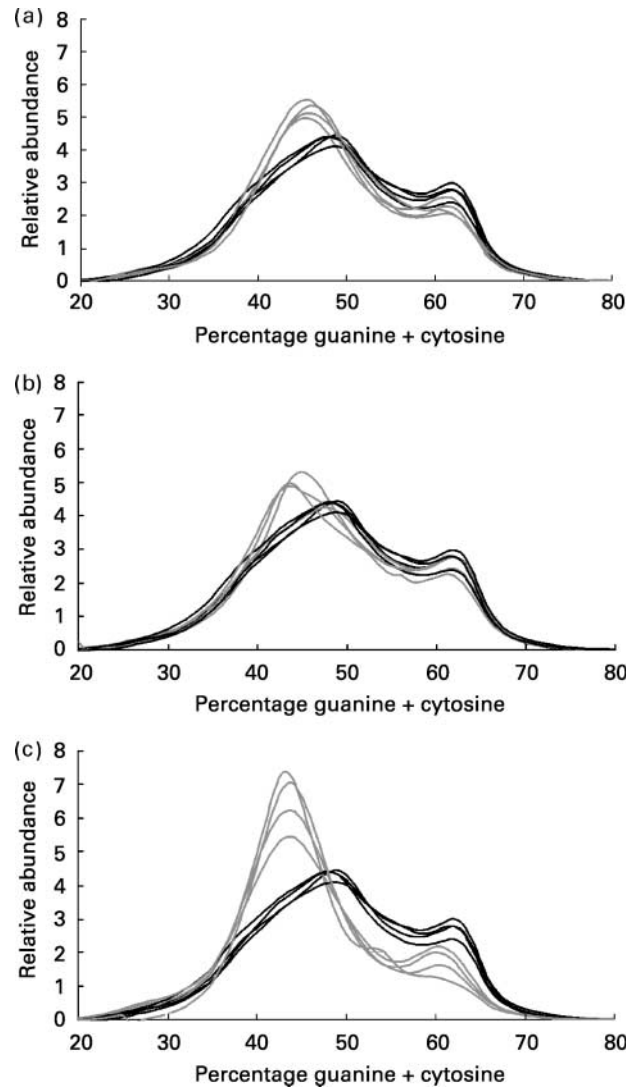
**Table 3.** Bacterial density in the caecum of rats fed on different diets (four samples per group)† (Mean values with their standard errors)

| Group        | Cell no. (per g digesta) |                   |
|--------------|--------------------------|-------------------|
|              | Mean                     | SE                |
| Control      | 1.02 <sup>11</sup>       | 5.37 <sup>9</sup> |
| PDX          | 8.06 <sup>10*</sup>      | 2.67 <sup>9</sup> |
| Lactitol     | 7.70 <sup>10*</sup>      | 7.09 <sup>9</sup> |
| PDX+lactitol | 8.68 <sup>10*</sup>      | 4.34 <sup>9</sup> |

PDX, polydextrose.

\*Mean value was statistically significantly different from that for the control ( $P < 0.001$ ).

† For details of diets and procedures, see p. 906.



**Fig. 1.** Effect of polydextrose (PDX), lactitol and their combination on the structure of the total microbial community in rat caecum. Chromosomal DNA of caecal bacteria was purified from individual rats and analysed by percentage guanine + cytosine profiling. (a), PDX diet; (b), lactitol diet; (c), PDX + lactitol diet; (—), rats fed on control diet; (---) rats fed on test diets.

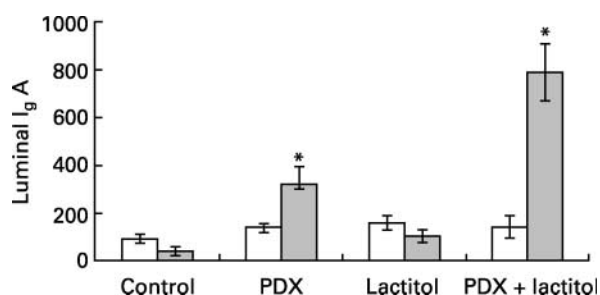
**Table 4.** Concentrations of biogenic amines in the caecum ( $\mu\text{M}$ ) of rats fed on different diets (twelve rats per group)\* (Mean values with their standard errors)

| Group...                  | Control |      | PDX   |      |               | Lactitol |      |               | PDX+lactitol |     |               |
|---------------------------|---------|------|-------|------|---------------|----------|------|---------------|--------------|-----|---------------|
|                           | Mean    | SE   | Mean  | SE   | $P < \dagger$ | Mean     | SE   | $P < \dagger$ | Mean         | SE  | $P < \dagger$ |
| Methylamine               | 173.5   | 14.3 | 118.5 | 6.7  | 0.0001        | 139.4    | 12.0 | 0.01          | 97.4         | 4.8 | 0.01          |
| Agmatine                  | 51.2    | 3.9  | 34.8  | 6.4  | 0.0001        | 61.1     | 4.2  |               | 32.1         | 2.0 | 0.0001        |
| Ethylamine                | 2.1     | 0.2  | 2.5   | 0.2  |               | 1.6      | 0.6  |               | 1.9          | 0.0 |               |
| Tryptamine                | 8.4     | 0.4  | 8.5   | 0.7  |               | 11.6     | 0.9  | 0.01          | 9.3          | 1.3 |               |
| Buthylamine               | 1.9     | 0.2  | 2.1   | 0.2  |               | 2.4      | 0.1  |               | 3.0          | 0.2 | 0.001         |
| $\beta$ -Phenylethylamine | 10.2    | 0.7  | 7.7   | 0.5  |               | 8.0      | 0.8  |               | 15.7         | 2.2 | 0.0001        |
| Putrescine                | 29.9    | 5.4  | 71.6  | 12.6 | 0.01          | 60.7     | 13.8 | 0.05          | 52.4         | 5.1 | 0.05          |
| Cadaverine                | 1.7     | 0.2  | 4.1   | 0.2  | 0.001         | 2.7      | 0.4  |               | 3.4          | 0.4 | 0.0001        |
| Histamine                 | 19.9    | 1.6  | 33.8  | 5.7  | 0.001         | 33.3     | 4.1  | 0.001         | 59.1         | 8.6 | 0.001         |
| Tyramine                  | 12.4    | 2.6  | 38.8  | 0.7  | 0.0001        | 4.8      | 3.5  | 0.01          | 27.9         | 3.5 | 0.01          |
| Spermidine                | 211.8   | 6.5  | 200.6 | 5.0  |               | 232.1    | 9.0  | 0.01          | 172.9        | 9.6 | 0.001         |
| Spermine                  | 19.5    | 1.0  | 20.9  | 3.0  |               | 33.8     | 2.0  | 0.0001        | 30.8         | 3.1 |               |
| Total                     | 1058    | 37   | 1025  | 46   |               | 1103     | 40   |               | 934          | 30  | 0.03          |

PDX, polydextrose.

\* For details of diets and procedures, see p. 906.

† Level of significance of difference from that for the control.



**Fig. 2.** Concentrations of luminal Ig A in the ileum ( $\square$ ) and caecum ( $\blacksquare$ ) of rats fed on different diets (twelve rats per group). Values are means, with their standard errors represented by vertical bars. \*Mean value was significantly different to that for the control group:  $P=0.007$  for polydextrose (PDX);  $P<0.0001$  for PDX+lactulose.

by 40% ( $P=0.02$ ). A similar increase was also seen in caecal TGF- $\beta$ -positive cells in combination with PDX ( $P=0.02$ ).

## Discussion

As a general rule carbohydrates are a preferred C source for bacteria. Therefore, simple carbohydrates may become

depleted before reaching the transverse colon. When this happens the main energy source in the distal (descending) colon is protein or complex carbohydrates (Cummings *et al.* 1987). Saccharolytic reactions can be characterised by the high production of acidic endproducts, resulting in the acidification of the colonic contents. In putrefactive reactions, basic endproducts are formed and therefore the pH of the lumen of the distal colon is closer to neutral. Putrefactive metabolic pathways produce branched SCFA,  $\text{NH}_3$ , biogenic amines and indoles (Macfarlane *et al.* 1986; Cummings & Macfarlane, 1991; Smith & Macfarlane, 1997). In the present study the tested compounds, i.e. PDX, lactitol and their combination, reduced the caecal pH of rats indicating caecal fermentation. Previous studies also show pH reduction by PDX and lactitol alone (Yoshioka *et al.* 1994; Piva *et al.* 1996; Hara *et al.* 2000). The most probable explanation for the observed pH reduction is bacterial fermentation of the carbohydrates.

PDX and lactitol reduced pH but had no significant effect on the residual SCFA concentrations in the present study. PDX at the concentration of 5% had a similar pH-lowering effect without a concomitant increase in SCFA concentrations in the study of Hara *et al.* (2000). They also observed that the volume of the caecum increased significantly with a

**Table 5.** Relative (%) area of cells producing cytokines interleukin-1, tumour necrosis factor- $\alpha$  and transforming growth factor (TGF)- $\beta$  in the ileal and caecal mucosa of rats fed on different diets (eight to nine rats per group)† (Mean values with their standard errors)

| Group...      |        | Control |      | PDX   |      | Lactitol |      | PDX+lactitol |      |
|---------------|--------|---------|------|-------|------|----------|------|--------------|------|
|               |        | Mean    | SE   | Mean  | SE   | Mean     | SE   | Mean         | SE   |
| IL-1          | Ileum  | 0.38    | 0.08 | 0.79* | 0.07 | 0.50     | 0.08 | 0.47         | 0.08 |
|               | Caecum | 1.13    | 0.26 | 1.18  | 0.25 | 1.03     | 0.14 | 1.23         | 0.18 |
| TNF- $\alpha$ | Ileum  | 0.65    | 0.09 | 0.75  | 0.13 | 0.79     | 0.13 | 0.76         | 0.17 |
|               | Caecum | 0.99    | 0.09 | 1.08  | 0.13 | 1.27     | 0.17 | 1.05         | 0.22 |
| TGF- $\beta$  | Ileum  | 0.63    | 0.13 | 0.51  | 0.11 | 0.64     | 0.13 | 0.44         | 0.13 |
|               | Caecum | 0.91    | 0.05 | 0.89  | 0.07 | 1.33*    | 0.13 | 1.18*        | 0.26 |

PDX, polydextrose.

\* Mean value was statistically significantly different from that for the control ( $P<0.05$ ).

† For details of diets and procedures, see p. 906.

PDX diet. One possible explanation for the pH reduction in the present experiment with PDX and lactitol is that the products of putrefaction in the digesta reduced even more. Indeed, the combination of PDX and lactitol significantly reduced the production of biogenic amines. Endo *et al.* (1991) observed that PDX supplementation in a Western diet reduced faecal pH and also decreased putrefactive biomarkers in human subjects. Mitsuoka (1996) saw a decrease in faecal putrefactive metabolites (p-cresol, indole, isobutyric and isovaleric acids), together with lower faecal pH. This can be considered beneficial since amines have been associated with a number of intestinal and systemic diseases (see Smith & Macfarlane, 1997).

In our control ('human') diet, the molar ratio of butyrate was approximately 10%, which is somewhat lower than that of 17% in the studies of Cheng *et al.* (1987) and Remesy & Demigne (1976). In the study of Goodlad & Mathers (1990) the molar concentration of butyrate was 14%. Lactitol alone significantly increased the residual butyrate molar ratio to 22% from 8% with the control diet. In the PDX+lactitol group the molar increase was also significant. Apajalahti *et al.* (2002) observed that soluble fibre, 10% inulin, did not increase butyrate production in mice with a semi-synthetic casein diet (molar ratio 3%), while in the starter diet (commercial rodent pellets) the butyrate molar ratio was 20%.

Butyrate is the preferred source of energy for colonocytes, which also improves the integrity of the mucosa, and appears to protect against colorectal cancer (Gamet *et al.* 1992; Smith *et al.* 1998; Abrahamse *et al.* 1999). A butyric acid analogue, tributyrin, is capable of inducing differentiation and enhancing apoptosis in malignant cells (Schroeder & Maurer, 2002). Piva *et al.* (2002) observed that tributyrin and lactitol had synergistic effects in the intestine of piglets. One notable observation was that these two compounds together decreased histamine levels in the jejunum and caecum, indicating a change in the putrefactive metabolism of pigs. Since PDX has been shown to increase butyric acid production in human subjects (Jie *et al.* 2000), combining it with lactitol could potentially enhance the integrity of the intestinal epithelium.

Based on the reduction of spermidine by lactitol and the PDX+lactitol combination, one could anticipate a reduction in the number of bacterial groups that produce biogenic amines, namely anaerobic cocci, fusobacteria and bacteroides (Noack *et al.* 1998). However, since no specific microbial groups were identified in the present study, the source of biogenic amines, whether originating from microbes or the animal itself (Seiler *et al.* 1998; Delzenne *et al.* 2000), remains speculative.

In the present study the total bacterial density in the caecum was reduced by all dietary treatments but PDX or lactitol should not directly inhibit colonic microflora. A lower bacterial density then hints towards the dilution of digesta and/or its faster transit in the gastrointestinal tract (see Govers *et al.* 1999). An enlargement of the caecal volume is one explanation for the reduced SCFA concentrations (for example, see Cheng *et al.* 1987). Caecal weight was not measured in the present study; an increase in weight would imply a larger volume and possibly

dilution of some of the measured compounds. The change in the caecal DM content in the present study was so small that it hardly explains the observed change. Digesta flow was not measured in the present study.

The studied compounds also had significant effects on the structure of the caecal microbial community as indicated by changes in the %G+C profiles. This method is useful since it provides a picture of the total microbial community and changes in response to diet and the environment (Apajalahti *et al.* 2001). The combination of PDX and lactitol had the strongest effect on the bacterial community composition. The major shift was seen in the bacteria that have 40–50% guanine and cytosine in their chromosomal DNA. Since several bacterial groups fit this description we cannot use this analysis for the identification of bacterial species. The study by Apajalahti *et al.* (2002) indicated that large shifts in the %G+C profiles occurred in the caeca of mice fed inulin compared with those fed a control diet. These changes were characterised by 16S rRNA sequencing and were found to be caused mainly by previously unknown bacterial species and also completely unknown bacterial genera. Therefore, without further analysis, speculation on the exact identity of microbes behind the changes in %G+C profiles in the present study is not warranted.

Even if the identity remains unknown, the changes in the microflora may have been related to immune stimulation observed in the present study. The increase in the %G+C area of 40–44 had a positive correlation with the increase in secretory Ig A ( $P < 0.01$ ). The presently observed connection between intestinal microbes and local immune responses is strengthened by the previous finding in which some species of *Lactobacillus*, also residing in the %G+C area of 40–44, stimulated Ig A production when added to the diet (Kaila *et al.* 1992). Secretion of Ig A can be induced also by supplementing the diet with fibre since PDX alone significantly increased Ig A production in the lower intestine while the combination of PDX+lactitol further increased this response almost ten-fold compared with the control. The mechanism behind this synergistic effect is unknown. The additional stimulation of Ig A secretion of the PDX and lactitol combination, in comparison with PDX alone, may be explained by the boost of energy (butyrate) for immune and epithelial cells provided by lactitol (see also Schely & Field, 2002) and/or by the ability of lactitol to induce TGF- $\beta$  production in mucosal cells. TGF- $\beta$ , among many other effects, promotes the switching of B cells to producing Ig A (Coffman *et al.* 1989).

In the present study there were no changes in the crypt:villus ratio of the rats fed on the different dietary treatments. The villus length:crypt depth ratio can be used as a sensitive indicator of product safety and it has been shown to be very sensitive to gastrointestinal pathogens (Larkin & Hannan, 1984; Keenan *et al.* 1986) and dietary changes (Southon *et al.* 1985; Sagher *et al.* 1991; Galluser *et al.* 1993). Interestingly, both the villus height and crypt depth reduced significantly when PDX, lactitol or their combination was added to the diet. High-viscosity carboxymethylcellulose reduced villus height in pigs while the low-viscosity counterpart increased

it (McDonald *et al.* 2001). Dirks & Freeman (1987) observed that 4.5% and 9.0% cellulose, pectin or hemicellulose increased villus height in rat jejunum or ileum. The significance of these differences is unclear at present. Yet, in the present study, no signs of inflammation were seen in the histomorphometric parameters.

Chronic intestinal inflammations are increasingly common, such as, for example, some food allergies or irritable bowel disease. Furthermore, a continuous inflammation in the colon is associated with an increased risk for colon cancer (Levin, 1992).

The T-lymphocytes secreting TGF- $\beta$  in the lower gastrointestinal tract are thought to be associated with the enhanced function of the epithelial barrier (Roche *et al.* 2000). Lactitol alone and in combination with PDX significantly increased the presence of these cells in the caecum. Since lactitol also appeared to stimulate butyric acid in the caecum, it seems justified to say that lactitol promoted the integrity of the epithelial barrier in the present study. The relative area of cells producing IL-1 was increased by the addition of PDX. IL-1 is generally considered as a pro-inflammatory cytokine; it can be detected in the intestinal mononuclear cells of patients with chronic intestinal inflammation (Olson *et al.* 1993). However, the role of IL-1 in the intestine is not clearly understood. IL-1 is secreted by normal intestinal tissue (Gionchetti *et al.* 1992). Furthermore, intestinal epithelial cells express IL-1 receptors (McGee *et al.* 1996) and IL-1 has been suggested to counteract non-steroid anti-inflammatory drug-induced mucosal damage in the gut (Gyires, 1994). We believe that the increase in IL-1-producing cells in the present study was not associated with inflammatory processes since the number of TNF- $\alpha$ -producing cells did not increase concomitantly and no indications of inflammation were seen in the tissue slices. Finally, non-inflammatory responses such as Ig A secretion were clearly induced. When assessing the role of dietary fibre on intestinal mucosa, it is clearly important to realise the presence of various types of immune responses and measure them accordingly.

In conclusion, PDX showed effects in rats that are associated with dietary fibre. The combination of PDX + lactitol caused changes in the rat gastrointestinal tract that were distinct from those caused by these molecules alone. Shifts in the microflora with an associated change in the microbial metabolism, including butyrate production and reduction in putrefaction, could possibly reduce the risk of colon carcinogenesis. Also, a very significant increase in the luminal Ig A concentration is an important indication of a potentially improved immune defence.

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