

Short-chain fatty acid formation in the hindgut of rats fed native and fermented oat fibre concentrates

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The formation of SCFA in rats fed fermented oat fibre concentrates was compared with that of rats fed native oat fibre concentrate. The cultures used were lactic acid bacteria consisting of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (V2), the exopolysaccharide-producing strain *Pediococcus damnosus* 2.6 (Pd) and *L. reuteri* (Lr). The materials were incorporated into test diets yielding a concentration of indigestible carbohydrates of 80 g/kg (dry weight). Rats fed the V2-fermented fibre-concentrate diet yielded higher caecal and distal concentrations of acetic acid ($P < 0.01$) than rats fed the native fibre concentrate. All the fermented fibre concentrates resulted in a higher propionic acid concentration in the distal colon ($P < 0.05$), while rats fed Pd-fermented fibre concentrate resulted in lower concentration of butyric acid ($P < 0.05$, $P < 0.01$) in all parts of the hindgut as compared with rats fed the native fibre concentrates. Butyrate concentrations ranged between 5–11 $\mu\text{mol/g}$ (distal colon) and 6–8 $\mu\text{mol/g}$ (13 d faeces). Higher proportions of acetic acid ($P < 0.05$; $P < 0.01$) were observed in the caecum of rats fed the fermented fibre concentrates. Rats fed Pd- and Lr-fermented fibre concentrates produced higher proportions of propionic acid ($P < 0.05$; $P < 0.01$) in the caecum. Changes in SCFA formation in the caecum, distal colon and faeces of rats fed the fermented samples compared with the native sample indicate that these microbes probably survive in the hindgut and that modification of the microflora composition with fermented foods is possible. This may be important for the gastrointestinal flora balance in relation to colonic diseases.

Short-chain fatty acids: Fermented oat fibre concentrate: Lactic acid bacteria: Rat hindgut

The colon is one of the least understood and most metabolically active organs (Eastwood, 1991). It is a tubular contractile reservoir that retains water, electrolytes, and ions and has a complex assemblage of micro-organisms that are responsible for the colon's degradative abilities. The main carbon and energy sources for colonic microflora are certain types of carbohydrates usually referred to as indigestible carbohydrates. The type and amount of endproducts formed by colonic bacteria depend on the chemical structure, composition and amounts of the available substrate, as well as the biochemical characteristics and catabolite regulatory mechanisms of the bacteria involved (Macfarlane & Macfarlane, 1997). The main endproducts from microbial degradation in the hindgut of dietary carbohydrates that escape digestion are SCFA, mainly acetic, propionic and butyric acid (Ruppin *et al.* 1980). Gases such as CO_2 , H_2 and CH_4 are also produced. There is increasing evidence that some SCFA have specific physiological effects. Acetic and propionic acid are quickly absorbed through the colonic mucosa, stimulating salt and water uptake (Ruppin *et al.* 1980). A high production of SCFA may also lower colonic pH, resulting in an increased mineral solubility and reduced formation of secondary bile acids (Nagengast *et al.* 1988; Delzenne *et al.* 1995). Further, the proliferation of unwanted pathogens may decrease as a

result of lower colonic pH. Butyric acid and to some extent propionic acid are important energy substrates for the colonocytes (Roediger, 1982), and butyric acid especially has been suggested to play a part in the prevention and treatment of ulcerative colitis (Cummings, 1997) and cancer (Scheppach *et al.* 1995). Studies *in vitro* have also shown that butyric acid may inhibit the growth of human cancer cells (Whitehead *et al.* 1986; Gamet *et al.* 1992) and can induce apoptosis (Hague *et al.* 1995) in colonic tumour cell lines. Propionic acid may lower plasma cholesterol concentrations by inhibiting cholesterol synthesis from acetic acid in the liver (Wolever *et al.* 1991) and also enhances colonic muscular contraction, relieving constipation and contributing to laxation (Yajima, 1985). Acetic acid promotes the relaxation of resistance vessels in the colonic vasculature, thus helping in the maintenance of blood flow to the liver and colon (Mortensen *et al.* 1990).

The amount and pattern of SCFA formed in the colon are highly dependent on the type of carbohydrate available. Some types of resistant starch, barley β -glucans, raffinose and oligofructose appear to promote butyric acid production both *in vitro* (Casterline *et al.* 1997; Karppinen *et al.* 2000) and in rats (Berggren *et al.* 1993; Mathers *et al.* 1997; Nilsson & Nyman, 2005). Arabinogalactans and guar gum result in

Abbreviations: EPS, exopolysaccharides; Lr, *Lactobacillus reuteri*; Pd, *Pediococcus damnosus* 2.6; V2, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

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high yields of propionic acid (Berggren *et al.* 1993; Edwards, 1993) in rats. Oat dietary fibre has been shown to result in a higher amount of butyric acid (1.44 mmol/g) by *in vitro* fermentation with human faecal inocula as compared with one-autoclaved cycle-resistant starch (0.94 mmol/g) (Casterline *et al.* 1997). The formation of SCFA is dependent on physico-chemical characteristics of the carbohydrate reaching the colon, such as monomeric composition, glycosidic linkages, molecular weight and solubility (Henningsson *et al.* 2001; Nilsson & Nyman, 2005), which in turn can be affected by heat treatment (Goodlad & Mathers, 1992) and fermentation.

Oat-based media could be suitable for the growth of lactic acid bacteria and also for the formation of microbial or exopolysaccharides (EPS) (Mårtensson *et al.* 2002a,b). A straight-chained β -(1 \rightarrow 3) glucan polymer with β -(1 \rightarrow 2) glucose monomers linked to the interior chain has also been isolated from *Pediococcus damnosus* 2.6 (Pd) (Dueñas-Chasco *et al.* 1997). This EPS can be produced in an oat-based medium after incubation with Pd (Mårtensson *et al.* 2002a,b). The production of EPS with α -(1 \rightarrow 3) glucan and α -(2 \rightarrow 6) fructan structures has also been observed in *Lactobacillus reuteri* (Lr) LB 121 growing on sucrose (van Geel-Schutten *et al.* 1999). However, there is yet no evidence that Lr ATCC 55 730 used in the present study produces EPS in an oat-based medium. EPS are capable of improving the texture and viscosity of the final product (Ricciardi *et al.* 1994) and may also improve the sensory and nutritional properties. The dairy industry uses some cultures that have been described as promoters of ropiness or mouthfeel because of their texture-enhancing properties (Sutherland, 1998; Folkenberg *et al.* 2005). Further, Nakajima *et al.* (1992) reported that there was a reduction in serum lipids in rats fed a ropy milk product containing a phospho-polysaccharide. The physiological effects of lactic acid bacteria-produced EPS may depend on their ability to resist degradation by gastrointestinal enzymes, and thus behave like a type of dietary fibre.

The objective of the present study was to evaluate the formation of SCFA along the hindgut of rats fed a β -glucan-enriched oat fibre concentrate fermented with different lactic acid bacteria. Commercial yoghurt strains consisting of a mixture of (a) *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* (V2), (b) Lr ATCC 55 730 and (c) the EPS-producing bacteria Pd were used in the fermentations of the oat fibre concentrate. The effects of the diets on food intake and weight gain, weight of the caecum contents, bulking effect and SCFA production were investigated.

Materials and methods

Bacterial strains

The probiotic culture Lr, the EPS-producing culture Pd and the commercial yoghurt strain, V2, were used. Lr was obtained from Biogaia Biologics (Stockholm, Sweden). Pd was received from the collection at the University of San Sebastian (Universidad del País Vasco, Spain). Pd and Lr strains were stored at -80°C in De Man–Rogosa–Sharpe broth (De Man *et al.* 1960) containing 25% (v/v) glycerol. The commercial yoghurt culture V2 was a 1:1 mixture of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* (Visby Tønder A/S, Tønder, Denmark); V2 was stored at -80°C according to the recommendation of the manufacturer. V2 was chosen because it is the starter culture commonly used in Sweden and improves aroma and acidity in the final product.

Preparation of the oat fibre concentrates

Oat fibre concentrate in powder form was obtained from Ceba Foods AB (Lund, Sweden). The powder was reconstituted to a DM of 10%, with aqueous distilled water. Glucose (2%, w/v) was added to the concentrate as an additional substrate for microbial growth and the mixture was sterilised at 80°C for 20 min. The concentrate was then cooled to fermentation temperature (28°C for Pd and 37°C for V2 and Lr) and inoculated. The fermentations were performed over a period of between 16 and 20 h (due to the different growth rate of the various lactic acid bacteria) under anaerobic conditions. The amount of V2 culture used as inoculum was 0.02% (w/v). Inoculates for Pd and Lr (5%, v/v) were taken from a fresh (20 h incubation) pre-inoculum in De Man–Rogosa–Sharpe broth (Merck, Darmstadt, Germany) and the cells were collected and washed with 0.9% (w/v) NaCl solution. The final pH of the fermented fibre concentrates was 4.2 ± 0.5 . All samples were lyophilised using a Labconco lyphlock 12 freeze-dry system (Ninolab AB, Väsby, Sweden) and milled to a particle size less than 0.3 mm in a Cyclotec mill (Tecator AB, Höganäs, Sweden). The chemical composition of the oat fibre concentrates is shown in Table 1.

Animals and experimental design

Male Wistar rats with an average weight of 91.0 (SEM 3) g were purchased from B&K Universal (Stockholm, Sweden). The rats were randomly divided into groups of seven in order to obtain

Table 1. Composition of oat fibre concentrates (g/100 g dry weight)

	Native	Fermented with V2	Fermented with Pd	Fermented with Lr
Protein	28.0	29.0	29.0	28.0
Lipids	14.0	13.0	14.0	13.5
Starch	21.4	21.0	22.3	23.3
Dietary fibre	25.0	24.5	24.8	25.3
Ash	2.6	2.4	3.0	1.2
NA*	9.0	10.1	6.9	8.7

V2, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; Pd, *Pediococcus damnosus* 2.6; Lr, *L. reuteri*;
NA, not analysed.

*Likely to be low-molecular-weight carbohydrates.

a similar average weight between the different test groups (91.0 (SEM 2.0), 90 (SEM 3.0), 90.5 (SEM 3.0), 92.8 (SEM 3.0) g for rats fed the native, V2-fermented, Pd- and Lr-fermented fibre concentrates respectively). The rats were housed individually in metabolism cages, with free access to water (Berggren *et al.* 1993). The feed intake was restricted to 12 g dry weight per d. After 7 d of adaptation to the diets, a 5 d experimental period followed when faeces were collected daily for the determination of faecal dry and wet weights, fermentability and bulking effect. Faeces were kept at -20°C and then freeze-dried and milled before analysis of total fermentability. Fresh faeces were collected on dry ice on day 6, to determine the faecal excretion of SCFA (13 d). At the end of the experiment, the animals were killed using CO_2 . The caecum and colon were immediately removed and the colon was divided into the proximal and the distal parts and kept frozen (-20°C) until analysed for SCFA. The caecal tissue, content weight and pH were measured before freezing the caecal content. The Ethics Committee for Animal Studies at Lund University (Sweden) approved the animal experiment.

Diets were formulated from four test products. These products were the native oat fibre concentrate, the V2-fermented oat fibre concentrate, Pd-fermented oat fibre concentrate and Lr-fermented oat fibre concentrate. All diets had a concentration of indigestible carbohydrates of 80 g/kg dry weight (corresponding to a total intake of about 4.8 g for each rat during the 5 d experimental period). Components of the test diets are shown in Table 2. The DM content of the diets was adjusted with wheat starch, a starch source that has been shown to be completely digested and absorbed in the upper intestinal tract and therefore does not contribute to any hindgut fermentation (Björck *et al.* 1987). The amount of SCFA excreted on a wheat starch diet has also been shown to be negligible (Berggren *et al.* 1993).

Analytical methods

Indigestible carbohydrates. The content of indigestible carbohydrates (dietary fibre) in the oat fibre concentrates was isolated in duplicate using the centrifugation–dialysis method (as described before in Lambo *et al.* 2005), in which molecules with a molecular weight ≥ 1000 Da will be recovered. The fibre values were corrected for remaining

proteins, lipids, starch and ash (Table 1). The monomeric composition of the dietary fibre in the isolated fibre residues and in faeces was analysed by GLC on a DB-225 column (J&W Scientific, Folsom, CA, USA) for the neutral sugars as their alditol acetates and spectrophotometrically for the uronic acids (Theander *et al.* 1995).

Crude protein, lipids, starch and minerals. The N was assayed by the Kjeldahl procedure (Kjeltec System 1003; Tecator AB, Höganäs, Sweden) according to the manual. Crude protein was calculated as $\text{N} \times 6.25$.

Total lipid content was determined gravimetrically by extraction in diethyl ether and petroleum ether (boiling point 40 – 60°C ; 1:1) after hydrolysis with 7.7 M-HCl at 70 – 80°C for 60 min (Association of Official Analytical Chemists, 1980).

Total starch in the fibre residues and faeces from the rats was quantified as described by Björck & Siljeström (1992). No starch could be detected in the faeces.

Ash content was determined by incineration at 550°C for at least 5 h, cooling in a desiccator and then weighing.

Determination of short-chain fatty acids. The amount of SCFA (acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, caproic, and heptanoic acid), lactic acid and succinic acid in the caecum, colon and 13 d faeces were analysed by GLC (Richardson *et al.* 1989). The intestinal content was homogenised (Polytron[®]; Kinematic AG, Lucerne, Switzerland) with 2-ethylbutyric acid as internal standard. Hydrochloric acid was added to protonise the SCFA, which were then extracted with diethyl ether and silylated with *n*-(tert-butyl)dimethylsilyl-*n*-methyltrifluoroacetamide (Sigma Chemical Company, MO, USA). The samples were allowed to stand for 24–48 h in order for complete derivatisation to occur and then they were analysed using an HP-5 column (Hewlett-Packard, Wilmington, DE, USA), and integrated by Chem Station software (Hewlett-Packard).

Calculations and statistical analyses

Bulking capacity of the indigestible carbohydrates was calculated as:

$$\frac{\text{Faecal dry weight}_{\text{test diet}} \text{ (g)}}{\text{Indigestible carbohydrates ingested}_{\text{test diet}} \text{ (g)}}$$

Table 2. Components of test diets (g/kg dry weight)

Component	Native	Fermented with V2	Fermented with Pd	Fermented with Lr
Dietary fibre source	320	320	320	320
Casein	80	80	80	80
Maize oil	10	10	10	10
Sucrose	100	100	100	100
DL-Methionine	1	1	1	1
Mineral mixture*	48	48	48	48
Vitamin mixture†	8	8	8	8
Choline chloride	2	2	2	2
Wheat starch	431	431	431	431

V2, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; Pd, *Pediococcus damnosus* 2.6; Lr, *L. reuteri*.

*Contained (g/kg): $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.37; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.4; KH_2PO_4 , 332.1; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 171.8; CaCO_3 , 324.4; KI, 0.068; MgSO_4 , 57.2; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 7.7; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 3.4; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.020; NaCl, 101.7.

†Contained (g/kg): menadione, 0.62; thiamine hydrochloride, 2.5; riboflavin, 2.5; pyridoxine hydrochloride, 1.25; calcium pantothenate, 6.25; nicotinic acid, 6.25; folic acid, 0.25; inositol, 12.5; *p*-aminobenzoic acid, 1.25; biotin, 0.05; cyanocobalamin, 0.00375; retinal, 0.187; vitamin D, 0.000613; vitamin E, 25; maize starch, 941.25.

Fermentability (%) of the indigestible carbohydrates was calculated as:

$$\left(1 - \frac{\text{Indigestible carbohydrates in faeces}_{\text{test diet}} (\text{g})}{\text{Indigestible carbohydrates ingested}_{\text{test diet}} (\text{g})}\right) \times 100.$$

The molar proportion of SCFA was calculated as a ratio of the total concentration of organic acids analysed to the individual acid concentration and expressed as a percentage.

All statistical analyses were performed with the Minitab[®] software package version 13 (Minitab Inc., State College, PA, USA). The means and standard deviations were analysed using the two-sided Student's *t* test. Significance of difference between the means of the fermented fibre concentrates and the native fibre concentrates was determined ($P < 0.05$ and $P < 0.01$).

Results

Monomeric composition of the indigestible carbohydrate

The monomeric composition of the indigestible carbohydrate in the native and fermented fibre concentrates is shown in Table 3. The main monomer in all materials was glucose. The distribution between the neutral sugars and uronic acids was quite similar in all the concentrates.

Basal data

The feed intake was almost complete and the rats gained weight throughout the experiment (Table 4). Body-weight gain, wet and dry faecal outputs, caecal contents, bulking capacity and fermentability were similar among the different groups and no significant differences could be seen.

Short-chain fatty acid concentrations

Acetic acid was the major metabolite in rats fed the oat fibre concentrates, followed by propionic and butyric acids. Approximately 80% of the organic acids analysed in the caecum of rats could be attributed to these acids. Some lactic acid could also be detected (Table 5).

A significantly higher concentration of acetic acid was observed in the caecum and distal colon of rats fed the V2-fermented fibre concentrate (51 and 29 $\mu\text{mol/g}$, respectively; $P < 0.01$) as compared with the native fibre concentrate (39 and 21 $\mu\text{mol/g}$, respectively) (Fig. 1).

The propionic acid concentrations were similar in rats fed the different fibre concentrates. The exception was in the distal colon of rats fed the fermented fibre concentrates and

the 13 d faeces of the rats fed the Pd-fermented fibre concentrate, where significantly higher ($P < 0.05$) concentrations of propionic acid were obtained, as compared with the native fibre concentrate (Fig. 1).

The caecal butyric acid concentrations in rats fed the Pd- (9 $\mu\text{mol/g}$) and Lr- (15 $\mu\text{mol/g}$) fermented fibre concentrates were significantly lower ($P < 0.05$) than in those fed the native fibre concentrate (24 $\mu\text{mol/g}$). In the proximal and distal colon, only the Pd-fermented fibre concentrate yielded a significantly lower ($P < 0.05$) butyric acid concentration as compared with the native fibre concentrate.

Proportions of short-chain fatty acids

Some differences were also observed in the proportions of SCFA among the rats fed the different fibre concentrates (Fig. 2). The rats fed the fermented fibre concentrates yielded significantly higher ($P < 0.05$; $P < 0.01$) proportions of acetic acid in the caecum than the native fibre concentrate. A higher proportion of acetic acid ($P = 0.014$) was formed in the distal colon of rats fed the Pd-fermented fibre concentrate as compared with the rats fed the native fibre concentrate.

Proportions of propionic acid were higher ($P < 0.05$; $P < 0.01$) in the caecum of rats fed the Pd- ($P = 0.043$) and Lr- ($P = 0.004$) fermented fibre concentrates than in the caecum of rats fed the native fibre concentrate. There was also a tendency for rats fed the Pd-fermented fibre concentrate to yield higher proportions of propionic acid in the distal colon and 13 d faeces as compared with the rats fed the native fibre concentrate (Fig. 2).

In the caecum, the rats fed the Pd- and Lr-fermented fibre concentrates produced significantly lower ($P < 0.05$; $P < 0.01$) butyric acid proportions than the rats fed the native fibre concentrate. In the distal colon, only the rats fed the Pd-fermented fibre concentrate had a significantly lower ($P = 0.010$) butyric acid proportion.

Caecal pools and faecal excretions

Caecal pool and faecal excretions of the organic acids are shown in Table 5. The caecal pool of organic acids (SCFA + lactic acid) in rats fed the V2-fermented fibre concentrate was higher ($P = 0.026$) than the caecal pool in rats fed the native fibre concentrate due to higher amounts of acetic ($P = 0.008$) and propionic acids ($P = 0.048$). Rats fed the Pd-fermented fibre concentrate had lower caecal pools of butyric acid ($P = 0.007$) and lactic acid ($P = 0.010$) than those fed native fibre concentrate. However, the faecal excretion of propionic acid was higher ($P = 0.017$) for rats fed the Pd-fermented

Table 3. Monomeric composition (%) of indigestible carbohydrates in the oat fibre concentrates

	Native	Fermented with V2	Fermented with Pd	Fermented with Lr
Arabinose	2.5	3.0	3.2	2.6
Xylose	2.5	2.5	2.7	2.0
Glucose	81.0	83.0	82.9	82.2
Uronic acids	11.4	10.2	9.1	10.2
Minor components*	2.6	1.3	2.1	3.0

V2, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; Pd, *Pediococcus damnosus* 2.6; Lr, *L. reuteri*.
*Rhamnose, fucose, mannose and galactose.

Table 4. Initial weight (g), feed intake (g/d) and body-weight gain (g/d) of rats, and faecal and caecal contents (g/d), fibre intake (g/d), bulking capacity and fermentability (%) in rats fed native and fermented oat fibre concentrates*

(Mean values with their standard errors for seven rats per group)

	Native		Fermented with V2		Fermented with Pd		Fermented with Lr	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Initial weight	91.0	2.0	90.0	3.0	90.5	3.4	92.8	3.0
Feed intake	11.4	0.7	11.3	0.8	11.6	0.2	11.6	0.4
Body-weight gain	3.8	1.0	3.1	1.4	4.0	1.4	3.5	1.0
Wet faeces	0.87	0.1	0.87	0.2	0.88	0.1	0.80	0.2
Dry faeces	0.54	0.02	0.60	0.03	0.60	0.06	0.53	0.07
Caecal content	2.3	0.1	2.5	0.6	2.0	0.3	2.5	0.5
Fibre intake	0.91	0.06	0.90	0.07	0.93	0.02	0.93	0.03
Bulking capacity	0.60	0.02	0.63	0.04	0.62	0.06	0.60	0.07
Fermentability	94.4	1.4	92.8	1.1	95.4	2.8	96.2	1.4

V2, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; Pd, *Pediococcus damnosus* 2.6; Lr, *L. reuteri*.

*No significant differences could be seen between the groups.

fibre concentrate as compared with those rats fed the native fibre concentrate.

Faecal excretions were higher for rats fed the Lr-fermented fibre concentrate (116 μmol ; $P=0.030$) as compared with those fed the native fibre concentrate (76 μmol). This could be explained by higher ($P<0.05$; $P<0.01$) excretions of acetic, propionic and lactic acids in rats fed this diet.

Discussion

SCFA, especially propionic and butyric acids, have been reported to have health-promoting properties and the desire to develop functional food products with the ability to improve health or prevent the incidence of certain diseases has become a challenge for the food industry. In the present investigation, oat fibre concentrate was fermented with different lactic acid bacteria and used in a rat experiment, in order to observe the SCFA formation in the hindgut. Oat fibre concentrates were fed to rats for 13 d, which has been reported to be sufficiently long to yield optimal fermentation of dietary fibre components (Nyman & Asp, 1985; Brunsgaard *et al.* 1995)

and a stable SCFA profile (Tulung *et al.* 1987; Levrat *et al.* 1991).

The total fermentability of the fibre was similar (about 92–96%) in the native and fermented oat fibre concentrates, and was in agreement with previous studies, which revealed values of about 90% for a β -glucan-enriched oat fibre (Henningsson *et al.* 2002) and about 97% for barley β -glucans (Berggren *et al.* 1993). Rats fed the V2-fermented fibre concentrate were more prone to form SCFA in the caecum, while those fed the Lr-fermented fibre concentrate resulted in the highest faecal excretion of SCFA, indicating a slower fermentation of the substrate with the presence of Lr (Table 5).

In the caecum and distal colon, the formation of acetic and propionic acids seemed to be favoured by the rats fed the fermented fibre concentrates, while butyric acid formation was comparatively higher in rats fed the native fibre concentrate. V2 and Pd have been observed to be homofermenters *in vitro* (convert glucose to lactic acid) (Axelsson, 1998; Battock & Azam-Ali, 1998), whereas Lr has been observed to be a heterofermenter (converts glucose to lactic and acetic acid) (Lindgren & Dobrogosz, 1990; Mitsuoka, 1992; Fujisawa *et al.* 1996). The colonic sugar fermenters *Clostridium* spp.,

Table 5. Caecal pool (μmol) and faecal excretion of organic acids (μmol)†

(Mean values with their standard errors for seven rats per group)

	Acetic acid		Propionic acid		Butyric acid		Lactic acid		Total pool	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Caecum										
Native	94	4	32	2	61	8	22	2	236	16
Fermented with V2	142***	12	54**	9	53	9	17	4	294**	15
Fermented with Pd	102	14	35	5	24***	6	11**	2	186	19
Fermented with Lr	112	22	43	6	36	12	11**	3	217	32
13 d Faeces										
Native	41	5	8	0.6	9	1	8	2	76	9
Fermented with V2	41	4	8	0.2	10	2	10	1	77	5
Fermented with Pd	53	6	13**	2	7	1	11	2	92	8
Fermented with Lr	61**	8	13**	2	12	4	20***	4	116**	18

V2, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; Pd, *Pediococcus damnosus* 2.6; Lr, *L. reuteri*.

†Mean values were significantly different from the corresponding value of the native fibre concentrate in the same part of the hindgut:

** $P<0.05$, *** $P<0.01$.

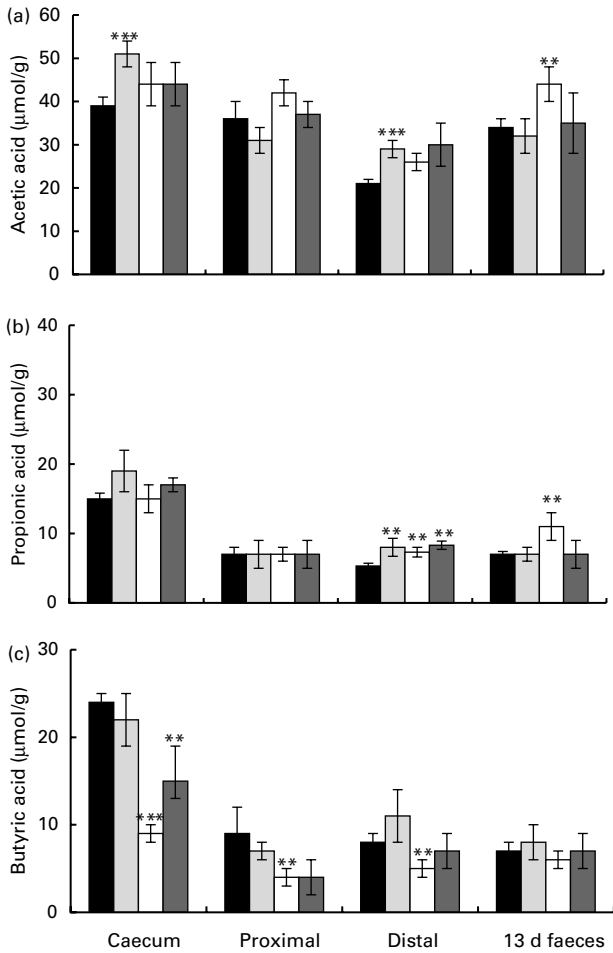


Fig. 1. Concentrations of (a) acetic acid, (b) propionic acid and (c) butyric acid ($\mu\text{mol/g}$ wet contents) in the caecum, proximal colon, distal colon and in 13 d faeces of rats fed native fibre concentrate (■), *Lactobacillus bulgaricus* and *Streptococcus thermophilus*-fermented fibre concentrate (□), *Pediococcus damnosus* 2.6-fermented fibre concentrate (□) or *L. reuteri*-fermented fibre concentrate (■). Data are means (n 7), with their standard errors represented by vertical bars. Mean values were significantly different from the corresponding value of the native fibre concentrate in the same part of the hindgut: ** $P < 0.05$, *** $P < 0.001$.

Eubacterium spp., *Fusobacterium* spp., *Butyrivibrio* spp. and acid utilisers, such as *Megasphaera elsdenii*, are all butyrate-producers (Holdeman *et al.* 1977; Tsukahara *et al.* 2002). Sugar fermenters produce butyric acid as the major end-product of fermentation, while the acid utilisers convert lactic acid to butyric acid (Tsukahara *et al.* 2002). *Bifidobacteria*, which are also present in the colon, produce both acetic and lactic acid. *Propionibacterium* sp. and *Veillonella* sp. are acetate and propionate producers (Mitsuoka, 1996; Ozadali *et al.* 1996). The production of butyric acid generated by acid converters such as *M. elsdenii*, for example, is stimulated by lactic and acetic acids (Tsukahara *et al.* 2002). This conversion process seems to depend on the rate at which lactic acid is produced. The varied amounts of acetic, propionic, butyric and the other organic acids observed in the present study most probably resulted from the metabolic activities of the microflora in the colon as well as from the ingested micro-organisms in the fermented fibre concentrates.

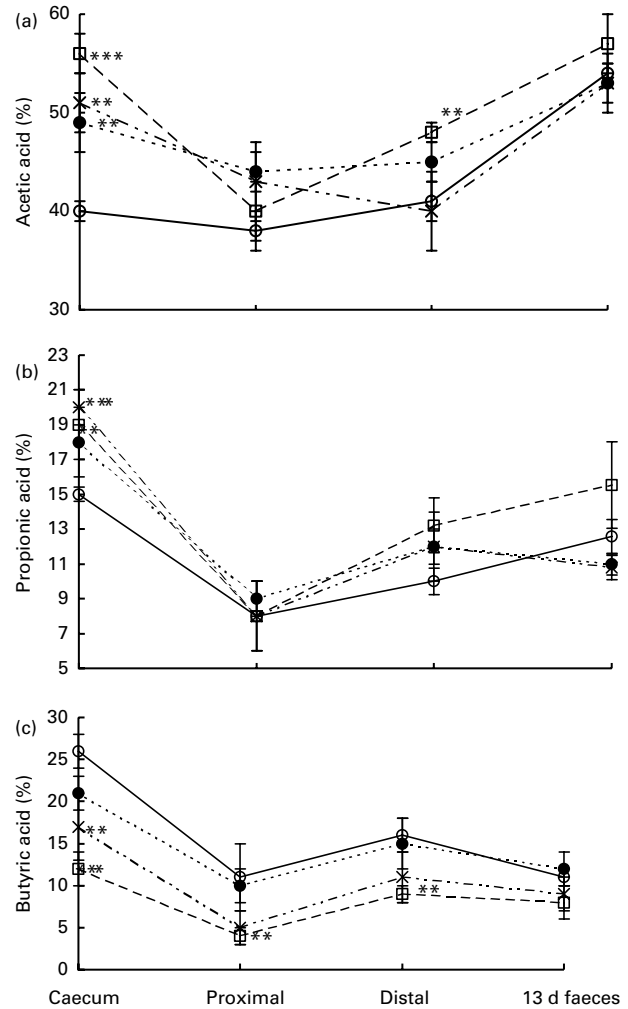


Fig. 2. Proportions of (a) acetic acid, (b) propionic acid and (c) butyric acid (percentage of total SCFA) in the caecum, proximal colon, distal colon and in 13 d faeces of rats fed native fibre concentrate (○), *Lactobacillus bulgaricus* and *Streptococcus thermophilus*-fermented fibre concentrate (●), *Pediococcus damnosus* 2.6-fermented fibre concentrate (□) or *L. reuteri*-fermented fibre concentrate (×). Data are means (n 7), with their standard errors represented by vertical bars. Mean values were significantly different from the corresponding value of the native fibre concentrate in the same part of the hindgut: ** $P < 0.05$, *** $P < 0.001$.

It may also be questioned if the ingested bacteria reach the hindgut of rats in a live state. There has been some controversy as to whether V2 survives passage through the intestinal tract. Bianchi-Salvadori *et al.* (1978) isolated viable V2 in the faeces of human subjects after yoghurt consumption, whereas Pedrosa *et al.* (1995) did not come to the same conclusion. In conventional rats, one of the micro-organisms in V2, *L. delbrueckii* spp. *bulgaricus* was not always present, whereas the other micro-organism, *S. salivarius* spp. *thermophilus* did not survive beyond the upper small intestine (Hargrove & Alford, 1978). *Lr.*, on the other hand, inhabits the gastrointestinal tract of mammals and hence survives the passage and reaches the colon (Casas & Dobrogosz, 2000), while there is yet no report about the survival of Pd *in vivo*. However, *in vitro* studies have shown that Pd can tolerate exposures to gastrointestinal juices (Immerstrand, 2005). The variations in the

formation of SCFA in the caecum and distal colon of rats fed the different fermented fibre concentrates could also be interpreted as a probable indication that there is survival of these microbes in the upper intestinal tract and that they reach the hindgut. When ingested, these micro-organisms might improve the balance of gastrointestinal flora by increasing the number and activity of endogenous bacteria possessing health-promoting properties.

The significantly higher concentration of propionic acid in the distal colon and the 13 d faeces, the 13 d faecal excretion and the tendency towards higher proportions of propionic acid in the distal colon and 13 d faeces of rats fed Pd-fermented fibre concentrate is evidence of the fact that this substrate persisted to a higher extent beyond the proximal colon as compared with the native fibre concentrate. This observed trend reveals that there was a tendency for propionic acid to be continuously formed to a higher degree in the distal part of the hindgut of rats fed Pd-fermented fibre concentrate than of rats fed the native fibre concentrate, which may be positive towards maintaining colonic health. Propionic acid (like butyric acid) is an important energy substrate for the colonic mucosa and, as most colonic diseases occur in the distal part of the colon, it is important to increase the formation of these acids distally. Furthermore, propionic acid has been suggested to inhibit the synthesis of cholesterol from acetic acid in the liver (Wright *et al.* 1990; Wolever *et al.* 1991). Interestingly, in a clinical study on human subjects, a rye oat-based product (fermented with the strains V2 and Pd 2.6) significantly reduced total cholesterol (4.3%) and increased faecal *Bifidobacterium* ssp. count compared with a fermented dairy-based product (Mårtensson *et al.* 2005). It could be speculated that this was due to an increased formation of propionic acid.

In the caecum, the proportion of butyric acid was lower ($P < 0.05$; $P < 0.01$) for rats fed the fermented (except for V2) products (12–21%) than for those fed the native fibre concentrate (26%). The values for the fermented products were, however, in a similar region as those observed from earlier studies (15%) for a β -glucan-enriched oat fibre and barley β -glucan, i.e. substrates known to promote comparatively high proportions of butyric acid (Berggren *et al.* 1993; Henningsson *et al.* 2002). The differences between the native and fermented fibre concentrates could probably be due to the added bacteria and their capability of modifying the composition of the microflora in the hindgut and thus the SCFA profile. Furthermore, possible changes in the biochemical characteristics of the substrates after *in vitro* fermentation could also contribute to the observed results. Thus, Lambo *et al.* (2005) observed that the physico-chemical properties, such as the amount of oats and barley β -glucans and viscosity of β -glucans solutions, were lowered after incubation with a mixture of lactic acid bacteria. However, in the present study the amount of dietary fibre including β -glucans was not lowered after fermentation, which may have been due to the fact that mixtures of lactic acid bacteria were not used in the fermentation. Furthermore, a higher amount of substrate (2% glucose) was also used in the present study as compared with 1% in the previous study. On the other hand, it cannot, however, be excluded that the molar mass of the dietary fibres and β -glucans were not modified during fermentation with the different lactic acid bacteria. Interestingly, Nilsson

& Nyman (2005) have shown that fructo-oligosaccharides with a low degree of polymerisation yielded higher amounts of butyric acid than those with a high degree of polymerisation, which instead yielded high amounts of propionic acid.

The proportions of butyric acid in the distal colon of rats fed the fibre concentrates (10–16%) were similar to earlier reports on potato products (14%) (Henningsson *et al.* 2002). A high formation of butyric acid in the colon, especially in the distal part, is interesting as it might protect against colon cancer (Whitehead *et al.* 1986; McIntyre *et al.* 1993; Hague *et al.* 1995; Thorup *et al.* 1995), and type III resistant starch has been found to play such a protective role in rats (Perrin *et al.* 2001). Colon cancer occurs mostly in the distal colon in both man and experimentally induced rodent cancer models (Bufill, 1990; Holt *et al.* 1996). Butyric acid may also have therapeutic effects in distal ulcerative colitis (Scheppach *et al.* 1992). A human study showed that β -glucan-enriched oat bran (20 g fibre) added to the diet of patients with ulcerative colitis for 12 weeks increased the concentration of butyric acid in the faeces by 36% and also improved the symptoms of the disease (Hallert *et al.* 2003). Furthermore, Perrin *et al.* (2001) observed that only those indigestible carbohydrates favouring a high and stable butyric acid production in the rat hindgut decreased the rate of aberrant crypt foci, one of the most reliable intermediate biomarkers of colon cancer (Pretlow *et al.* 1991, 1992; Konstantakos *et al.* 1996; Young *et al.* 1996; Shivapurkar *et al.* 1997).

Prebiotic products that maintain SCFA formation (even in small concentrations) in the distal colon are recommended. Butyric acid concentrations of 1–5 $\mu\text{mol/g}$ (Scheppach *et al.* 2001) have been found to effectively suppress cell proliferation in cultured colonocytes, but it is still unclear how these concentrations relate to conditions *in vivo*. This will probably depend on the extent of butyric acid uptake along the colon and the persistence of an active concentration (Robertson *et al.* 2001). Butyrate concentrations in the present study ranged between 4 and 9 $\mu\text{mol/g}$ in the proximal colon, 5 and 11 $\mu\text{mol/g}$ in the distal colon and 6 and 8 $\mu\text{mol/g}$ in the 13 d faeces. This implies that butyric acid formation persisted along the hindgut.

In conclusion, the fermented fibre concentrates seemed to favour the formation of acetic and propionic acids, while the native fibre concentrate favoured the formation of butyric acid.

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