Short-chain fatty acid formation in the hindgut of rats fed oligosaccharides varying in monomeric composition, degree of polymerisation and solubility

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The contents of short-chain fatty acids were investigated in rats fed lactitol, lactulose and four fructo-oligosaccharides of different degree of polymerisation and solubility. Fructo-oligosaccharides with a low degree of polymerisation (2–8) generated the highest levels of butyric acid all along the hindgut, whereas fructo-oligosaccharides with a high degree of polymerisation (10–60) generated the highest levels of propionic acid. These specific differences were also generally reflected in the caecal pools and molar proportions of short-chain fatty acids. The lower solubility of the fructo-oligosaccharides was related to a lower degree of caecal fermentation. Lactulose and lactitol yielded high proportions of acetic acid and low proportions of butyric acid. It is concluded that both the degree of polymerisation and the solubility may affect short-chain fatty acid formation, whereas the fructose content *per se* seem to be of less importance. This may be of interest when designing foods with specific health effects.

Short-chain fatty acids: Fructo-oligosaccharides: Lactulose: Lactitol

When indigestible carbohydrates are fermented in the human colon, short-chain fatty acids (SCFAs; mainly acetic, propionic and butyric acids) and gases (CO_2, CH_4 and H_2) are formed. Several studies have shown that some of these acids have positive effects on health, one of the most important effects being the trophic effect on the intestinal epithelium, through a stimulation of blood flow and mucosal proliferation, and thus a decrease in the risk of mucosal damage (Mortensen *et al.* 1990, 1991). Butyric acid, and to some extent also propionic acid, is an important energy substrate for the colonic mucosa (Roediger, 1980; Cummings & Macfarlane, 1991). It has therefore been suggested that these acids increase the resistance to diseases in the colon, such as ulcerative colitis (Cummings, 1997) and colonic cancer (Scheppach *et al.* 1992). Furthermore, butyric acid can inhibit the proliferation of colon cancer cells *in vitro* (Scheppach *et al.* 1995). Propionic acid has also been reported to have metabolic effects, through inhibiting hepatic cholesterol synthesis from acetic acid, and the higher the propionic:acetic acid ratio, the more beneficial the effects (Wolever *et al.* 1991). Usually, only the formation of acetic, propionic and butyric acid during fermentation is reported, but other SCFA are also formed. Some of these acids, for example the branched acids that are formed by the fermentation of indigestible proteins and not from carbohydrates (Salminen *et al.* 1998), have been associated with negative effects.

All SCFA have a lowering effect on pH, which may be beneficial as such. For example, mineral complexes that are insoluble at a physiological pH may be partly solubilised at a lower pH and therefore more easily absorbed through the colonic mucosa (Younes *et al.* 1996). The motility of the colon may also be influenced to some extent by SCFA, but other parts of the gastrointestinal tract are probably more affected (Cherbut *et al.* 1997). Furthermore, a lower pH is known to inhibit the bacterial transformation of primary to secondary bile acids (Nagengast *et al.* 1988).

Different types of indigestible carbohydrate have been shown to produce different SCFA patterns (Brighenti *et al.* 1989; Berggren *et al.* 1993; Casterline *et al.* 1997). One explanation of this could be differences in the monomeric composition of the carbohydrates. For example, both resistant starch fractions and β-glucans have been shown to provide high amounts of butyric acid, whereas plant material containing a high amount of pectins seems to be a particularly good source of acetic acid (Brighenti *et al.* 1989; Berggren *et al.* 1993). Results, however, are not conclusive, and other factors also seem to be of importance, for example the complexity of the food matrix and a combination of substrates (Henningsson *et al.* 2002). Furthermore, the types of linkage may have an effect, and β-glucans and resistant starch have been shown to give rise to different SCFA profiles when using the same *in vitro* system (Casterline *et al.* 1997).

Another explanation might be the degree of polymerisation (DP). Fructo-oligosaccharides have been shown to form high amounts of butyric acid in several studies on rats (Casterline *et al.* 1997; Le Blay *et al.* 1999). However, in one study, large amounts of propionic acid were reported (Levrat *et al.* 1991). As there are indications that fructo-oligosaccharides with

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a longer chain (inulin) were used in the latter study, it could be speculated that this was due to the higher DP of inulin. The site of fermentation has also been shown to be different for different types of carbohydrate, and more resistant fibres seem to be fermented more in the distal part of the colon (Henningsson *et al.* 2002). This is interesting as most colonic diseases also occur at this site.

The aim of the present investigation was to study the distribution and content of SCFA formed in various parts in the hindgut of rats given various indigestible carbohydrates with a different DP. The amounts of lactic acid and succinic acid were also quantified. For this purpose, four types of fructo-oligosaccharide were chosen for the study: oligofructose with a DP between 2 and 8, inulin with an average DP of 23 (10–60), which was also available in a variant that had a lower solubility, and finally a mixture of oligofructose and inulin. The fructo-oligosaccharides chosen were extracted from chicory root, and the polymers consisted of fructose monomers with β-2,1 glycosidic linkages that were not hydrolysed by human digestive enzymes (Franck, 2002). A fructose-containing disaccharide (lactulose, galactose and fructose with β-1,4 linkages) was also included in the study, as was lactitol (galactose and glucitol with β-1,4 linkages) as it has a composition similar to that of lactulose. Both these disaccharides reach the colon, where they are fermented.

Materials and methods

Materials

Fructo-oligosaccharides derived from chicory root with a different DP were obtained from ORAFTI (Tienen, Belgium): Raftilose P95 (oligofructose; OF) consisting of oligofructose with a DP between 2 and 8, Raftiline HP (long-chain inulin; IN) and Raftiline HPX (long-chain inulin with a low solubility; IN-ls), with a somewhat higher molecular weight, i.e. an average DP of 23 (10–60) (Franck, 2002). Raftiline HPX has a lower solubility (1 g/l at 25°C) than Raftiline HP (20 g/l at 25°C) and a higher gel strength in fat substitution applications according to the manufacturer's product sheets (ORAFTI).

Raftilose SYNERGY1, a mixture of OF and IN (1:1; Mix OF-IN), was also included; this consisted of a mixture of DP 10–60 and DP 2–8. OF is produced by the partial enzymatic hydrolysis of naturally occurring inulin extracted from the chicory root, whereas IN is obtained by removing fructo-oligosaccharides of a low DP from natural inulin (Franck, 2002). By modifying the powder characteristics in the subsequent spray-drying process, an inulin with lower solubility (IN-ls) and different functionality is obtained.

Two disaccharides were also investigated: lactulose, which is a disaccharide consisting of fructose and galactose (Calbiochem, Darmstadt, Germany), and lactitol, which consists of galactose and glucitol (Danisco, Copenhagen, Denmark).

Animals and diets

Male Wistar rats, 3–4 weeks old, were used in the experiments, seven in each group. One group of rats was fed a control diet containing no indigestible carbohydrates (Table 1). The control diet contained casein (Sigma Chemical Company, St Louis, MO, USA) as protein source, sucrose (Danisco Sugar, Malmö,

Table 1. Composition of test diets (g/kg dry weight)

Component	Control	Test diets
Fructo-oligosaccharides, lactitol, lactulose	–	80–86·9§
Casein	120	120
DL-Methionine	1·2	1·2
Maize oil	50	50
Mineral mixture†	48	48
Vitamin mixture‡	8	8
Choline chloride	2	2
Sucrose	100	100
Wheat starch	670·8	583·9–590·8‖

† Containing (g/kg) 0·37 CuSO₄.5H₂O, 1·4 ZnSO₄.7H₂O, 332·1 KH₂PO₄, 171·8 NaH₂.PO₄.2H₂O, 324·4 CaCO₃, 0·068 KI, 57·2 MgSO₄, 7·7 FeSO₄.7H₂O, 3·4 MnSO₄.H₂O, 0·020 CoCl.6H₂O, 101·7 NaCl.

‡ Containing (g/kg) 0·62 menadion, 2·5 thiamine hydrochloride, 2·5 riboflavin, 1·25 pyridoxine hydrochloride, 6·25 calcium pantothenate, 6·25 nicotinic acid, 0·25 folic acid, 12·5 inositol, 1·25 *p*-aminobenzoic acid, 0·05 biotin, 0·00375 cyanocobalamin, 0·187 retinol palmitate, 0·00613 calciferol, 25 d-α-tocopheryl acetate, 941·25 maize starch.

§ Purity of the oligosaccharides 92–100 % (dry weight).

‖ Depending on the purity of the added oligosaccharide.

Sweden), maize oil (Mazola, Bestfoods Nordic A/S, Copenhagen, Denmark), DL-methionine (Sigma Chemical Company), choline chloride (Aldrich Chemie, Steinheim, Germany), a mineral mixture (Apoteket, Malmö, Sweden), a vitamin mixture (Apoteket, Malmö, Sweden) and wheat starch (Lundbergs, Malmö, Sweden). Wheat starch can be expected to be completely digested and absorbed, and does not contribute to any hindgut fermentation (Björck *et al.* 1987). In the test diets, the oligosaccharides were substituted for wheat starch to a level of 80 g indigestible carbohydrate/kg diet (dry weight; Table 1).

The feed intake was restricted to 12 g dry weight/d, and water was given *ad libitum*. The rats were allowed 7 d to adapt to the diet, which was followed by a 5 d long experimental period during which faeces and feed residues were collected daily. The faeces were stored at −20°C and then freeze-dried and milled before being analysed for any remaining indigestible carbohydrates. The animals were killed using CO_2 narcosis, and the caecum and proximal and distal colon were removed. Caecal tissue weight, content and pH were measured directly, and the different parts of the hindgut were frozen and stored at −40°C until analysis.

The Ethics Committee for Animal Studies at Lund University approved the animal experiments.

Analysis

Carboxylic acids. A GLC method was used to analyse the amount of SCFAs (formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic, heptanoic acids). Other carboxylic acids (CAs) quantified with that method were lactic acid and succinic acid (Richardson *et al.* 1989). The intestinal content and the faecal samples were homogenised (using a Polytron; Kinematica, Luzern Switzerland) together with an internal standard (2-ethylbutyric acid; Sigma Chemical Company). Hydrochloric acid was added to protonise the SCFAs and to be able to extract them in diethylether. After being silylated with *n*-(tert-butyldimethylsilyl)-*n*-methyltrifluoroacetamide (Sigma Chemical Company), the samples were allowed to stand for 48 h to complete derivatisation. Samples were then injected onto an HP-5 column (GLC, HP 6890; Hewlett Packard, Wilmington, DE, USA). Chem Station software (Hewlett Packard) was used for the analysis.

Indigestible carbohydrates. The amounts of fructo-oligosaccharides, lactitol and lactulose in the raw material were suggested to be as determined by the manufacturers. The amount of fructo-oligosaccharide in the faeces was first estimated enzymatically (number 139106; Boehringer Mannheim, Mannheim Germany) after hydrolysis in perchloric acid (0·6 M, 15 min, 80°C) (Nilsson & Björck, 1988). Only rats fed IN and IN-ls had detectable amounts of fructose in their faeces. The amounts of fructo-oligosaccharide in the faeces from these rats were therefore also quantified with a more reliable method, the AOAC method 999·03 (McCleary *et al.* 2000).

In this method, fructo-oligosaccharides are treated with fructanase (exo-inulinase), and the amount of fructose is quantified with the R-hydroxy-benzoic acid hydrazine reducing-sugar method. Lactitol and lactulose in the faeces were extracted in ethanol (1:1, v/v) for 30 min at room temperature according to a previously developed method (Ekvall *et al.* 2005). Arabinose was used as the internal standard. The sugars were then analysed by high-performance anion-exchange chromatography with pulsed amperometric detection (Dionex 500; Dionex, Sunnyvale, CA, USA), and NaOH (15–200 mmol) was used as the eluent with a flow rate of 1·0 ml/min.

Calculations and statistical evaluation. The design of the experiment resulted in one control group and six test diets containing the different indigestible carbohydrates. All analyses were performed at least in duplicate. The maximum error of the analyses was less than 5 %.

DM digestibility was calculated as:

$$1 - \frac{\text{g dry matter in faeces}}{\text{g dry matter–ingested}}.$$

The caecal pools of CAs were calculated as the levels of each acid (µmol/g) multiplied by the weight of the caecal content. The values were extrapolated to a complete intake of indigestible carbohydrates (4·8 g) and thus corrected for the small amount of feed residue.

Minitab statistical software (Release 13·32 Minitab Inc. State college, PA, USA) was used for statistical evaluation of the results, and a general linear model (ANOVA) followed by Tukey's procedure for multiple comparisons was used to find significant differences ($P < 0.05$) between means. The results in Fig. 1 were evaluated with the two-sample student's *t* test ($P < 0.05$).

Results

Weight gain, feed intake, caecal pH and faecal weight

The feed intake was almost complete (91–100 %), and the gain in body weight differed only for the rats fed OF, which exhibited a higher weight gain than rats fed lactitol, lactulose or IN-ls (Table 2). Adding indigestible carbohydrates to the control diet increased the caecal content from 0·8 g to a mean of 2·3 SE 0·1 g ($P < 0.05$). The caecal content was highest for rats fed lactitol (2·8 g) and lowest for those fed IN-ls (1·8 g). Rats fed the other test diets had intermediate caecal contents (2·1–2·3 g). The caecal pH of rats given the control diet was 7·0. Mix OF-IN resulted in the most accentuated fall in pH, to about 6·4, whereas the other oligosaccharides resulted in a pH between 6·6 and 6·9 (Table 2).

The faecal fresh weight was highest in rats fed IN and IN-ls: 3·7 and 4·1 g/5 d *v.* mean 2·8 SE 0·2 g/5 d and 1·7 g/5 d ($P < 0.05$) for the other test diets and the control diet, respectively
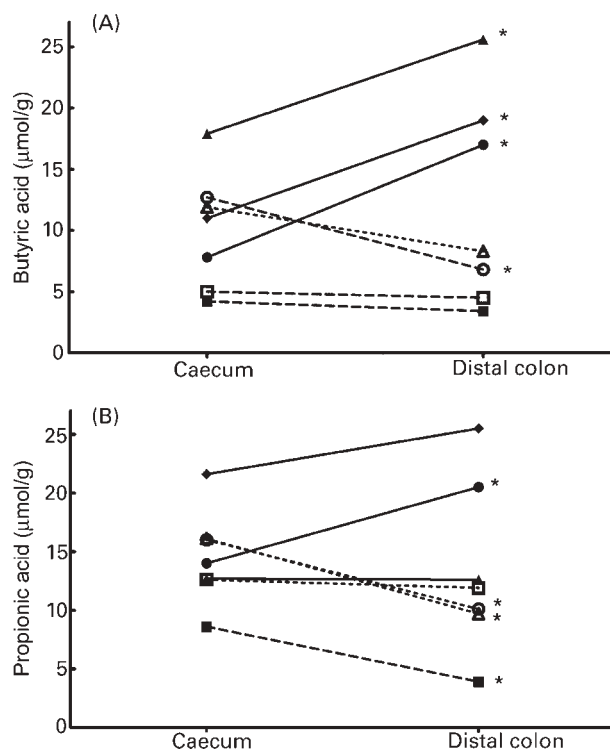
Fig. 1. Level (µmol/g wet content) in the caecum and distal colon of rats: (A) butyric acid and (B) propionic acid. (■) Control, (□) lactitol, (△) lactulose, (▲) oligofructose, (○) mixture of oligofructose and long-chain inulin, (◆) long-chain inulin, and (●) long-chain inulin with low solubility. *Levels in the distal colon are significantly different from those in the caecum ($P < 0.05$).

(Table 2). Thus, the bulking index (i.e. the fresh faecal increment in g/g indigestible carbohydrate ingested) was 0·4 and 0·5 for IN and IN-ls, respectively, *v.* 0·2–0·3 in rats fed the other test diets (data not shown). The faecal dry weight was also higher for rats fed IN (2·7 g/5 d) and IN-ls (3·0 g/5 d), but only the result for IN-ls was significant ($P < 0.05$), compared with rats fed lactitol and OF (2·1 g/5 d and 2·2 g/5 d, respectively; Table 2). The faecal dry weight increment per g ingested indigestible carbohydrate was on average 0·3 for IN-ls and IN in comparison with a mean of 0·1 for lactitol and OF (data not shown).

The DM digestibility decreased when fructo-oligosaccharides were added to the diet ($P < 0.05$), whereas lactitol and lactulose gave DM digestibility values similar to those of the control group. Rats fed IN-ls also showed a lower DM digestibility than rats fed lactulose and lactitol ($P < 0.05$). IN-ls was somewhat more resistant to fermentation than IN, as judged by the higher faecal excretion of fructo-oligosaccharides with IN-ls (0·44 g/5 d compared with 0·07 g/5 d g with IN; data not shown).

Carboxylic acids in the hindgut of rats

Caecal pool of carboxylic acids. The caecal pool of CAs was 2–4 times higher in the rats fed the test diets than in those fed the control diet without any indigestible carbohydrates (Table 3). There was, however, considerable variation between the groups fed the different test diets (97–188 µmol). The major acids formed were acetic acid (51–94 µmol), propionic acid (23–48 µmol) and butyric acid (9–43 µmol), these acids accounting for 81–93 % of the total amount of CAs analysed.

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Table 2. Feed intake, body weight gain, caecal content and pH, faecal weights and DM digestibility (DMD) in rats fed a control diet and test diets containing lactulose and lactitol and fructo-oligosaccharides with various degrees of polymerisation (DP) and solubility

(Values are means with their standard errors for seven rats per diet)

Diet	Feed intake (g/d)		Body weight gain (g/5 d)		Caecal content (g)		Faecal fresh weight (g/5 d)		Faecal dry weight (g/5 d)		DMD		Caecal pH	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	11·6	0·4	19·8[ab]	1·4	0·8[a]	0·0	1·7[a]	0·2	1·5[a]	0·1	0·974[a]	0·002	7·0	0·1
Lactitol	11·3	0·4	16·9[a]	1·8	2·8[b]	0·6	2·7[ab]	0·4	2·1[ab]	0·2	0·967[ab]	0·004	6·6	0·3
Lactulose	11·6	0·2	16·6[a]	1·7	2·3[b]	0·2	3·0[ab]	0·2	2·4[bc]	0·2	0·966[ab]	0·002	6·6	0·2
OF	10·9	0·5	26·6[b]	2·0	2·2[b]	0·3	3·0[ab]	0·4	2·2[ab]	0·2	0·960[bc]	0·003	6·6	0·1
Mix OF-IN	11·6	0·4	19·1[ab]	2·1	2·1[ab]	0·1	2·8[ab]	0·4	2·4[bc]	0·2	0·963[bc]	0·005	6·4	0·3
IN	11·8	0·2	20·8[ab]	1·5	2·2[b]	0·4	3·7[b]	0·3	2·7[bc]	0·2	0·955[bc]	0·003	6·6	0·3
IN-ls	12·0	0·0	18·9[a]	1·4	1·8[ab]	0·2	4·1[b]	0·3	3·0[c]	0·2	0·950[c]	0·003	6·9	0·1

OF, Raftilose P95 (ORAFTI) fructo-oligosaccharides with a DP between 2 and 8. IN and IN-ls, Raftiline HP and Raftiline HPX, both with an average DP of 23 (10–60), in which IN-ls has a lower solubility. Mix OF-IN, Raftilose SYNERGY1, a mixture of DP 2–8 (OF) and 10–60 (IN) (1:1).
[a,b,c]Mean values within a column with unlike superscript letters were significantly different ($P<0.05$).

The remaining part could be attributed to lactic acid (1–5 %), succinic acid (2–10 %) and isobutyric acid (1–2 %; data not shown).

Rats fed lactitol and IN-ls had a lower caecal pool of CA (mean 97 and 98 μmol, respectively) than rats fed the other test diets (173 SE 9 μmol; $P<0.05$). This was due to a lower content of all three main SCFA, and as a consequence these two substrates showed the lowest amount of propionic (mean 24 μmol) and butyric acid (mean 10 μmol). Furthermore, the caecal pool of butyric acid was higher in rats fed OF (43 μmol) than in those fed the disaccharides and the other fructo-oligosaccharides (9–27 μmol; $P<0.05$), whereas IN and Mix OF-IN had the highest caecal pools of propionic acid (46 and 48 μmol v. 23–33 μmol with the other test diets).

Levels of carboxylic acids in the hindgut. Rats fed lactulose had the highest levels of CAs in the caecum (90 μmol/g), whereas rats fed lactitol and IN-ls had the lowest levels (53 μmol/g and 60 μmol/g, respectively; $P<0.05$; Table 4). The highest level of butyric acid was found in rats fed the diet containing OF (18 μmol/g v. 5–13 μmol/g for the other test diets; $P<0.05$), whereas the highest level of propionic acid was found in rats fed the IN diet (22 μmol/g v. 13–16 μmol/g for the other test diets; $P<0.05$). Lactulose was found to generate the highest amount of acetic acid in the caecum of rats (45 μmol/g), which was significantly higher than the value for rats fed the other test diets except IN.

Similar tendencies could generally be seen in the proximal and distal colon (Table 4), with lactulose giving the highest level of acetic acid, OF the highest level of butyric acid and IN the highest level of propionic acid.

There was a higher level of butyric acid in the distal part of colon than in the caecum of rats fed OF, IN and IN-ls ($P<0.05$), whereas it decreased or was similar with the other test diets (Fig. 1 (A)). With regard to propionic acid, the level increased in groups fed IN and IN-ls ($P<0.05$; Fig. 1 (B)).

Low caecal levels of lactic acid (1–4 μmol/g) and succinic acid (2–9 μmol/g) were also identified, for which lactulose, OF and Mix OF-IN produced the highest amount of succinic acid (6–9 μmol/g; Fig. 2). Only small amounts of formic acid, isobutyric acid, isovaleric acid, valeric acid, caproic acid and heptanoic acid (0·1–1·1 μmol/g) could be found in caecum (Fig. 3).

Distribution of acetic, propionic and butyric acid in hindgut. The highest molar proportions of propionic acid in the caecum were seen in rats fed IN (30 %, v. a mean of 25 ± 1 % for the other oligosaccharides), whereas rats fed OF generated the highest proportions of butyric acid (31 %. v. 15 SE 2 % for rats fed the other substrates; $P<0.05$; Table 4). These differences were also seen in the colon. High proportions of butyric acid were, however, also seen in the distal part of the colon ($P<0.05$) of rats fed IN+ls and IN. Furthermore, rats fed lactulose generally had rather low proportions of propionic acid and butyric acid, and

Table 3. Caecal pools (μmol) of carboxylic acids in rats fed a control diet and test diets containing lactulose and lactitol and fructo-oligosaccharides with various degrees of polymerisation (DP) and solubility

(Values are means with their standard errors for seven rats per diet)

Diet	Acetic acid		Propionic acid		Butyric acid		Lactic acid		Total	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	26·6[a]	2·2	6·6[a]	0·5	3·2[a]	0·4	2·1[a]	0·3	42·3[a]	3·5
Lactitol	55·1[ab]	4·6	24·6[b]	1·2	9·3[a]	0·7	1·5[a]	0·2	97·4[ab]	5·0
Lactulose	93·8[c]	9·9	33·2[bc]	3·1	24·9[bc]	3·6	3·8[ab]	0·5	187·7[c]	17·4
OF	74·0[bc]	12·4	31·9[bc]	3·7	42·7[d]	4·8	1·8[a]	0·8	171·5[c]	21·1
Mix OF-IN	76·3[bc]	9·4	47·7[c]	9·7	27·0[c]	3·0	8·1[b]	3·1	176·4[c]	21·1
IN	74·6[bc]	7·0	45·7[c]	5·1	24·4[bc]	2·7	3·7[ab]	0·9	155·2[bc]	13·3
IN-ls	51·3[ab]	2·5	22·9[b]	1·3	12·9[ab]	0·9	2·1[a]	0·4	97·9[b]	4·3

OF, Raftilose P95 (ORAFTI) fructo-oligosaccharides with a DP between 2 and 8. IN and IN-ls, Raftiline HP and Raftiline HPX, both with an average DP of 23 (10–60), in which IN-ls has a lower solubility. Mix OF-IN, Raftilose SYNERGY1, a mixture of DP 2–8 (OF) and 10–60 (IN) (1:1).
[a,b,c]Mean values within a column with unlike superscript letters were significantly different ($P<0.05$).

Table 4. Levels of carboxylic acids (μmol/g wet content) and distribution between acetic, propionic and butyric acids (%) in different parts of the hindgut of rats fed diets containing lactulose, lactitol and fructo-oligosaccharides with various degrees of polymerisation (DP) and solubility

(Values are means with their standard errors for seven rats per diet)

| | Level (μmol/g) | | | | | | | | Proportion (%) | | | | | |
| | Acetic acid | | Propionic acid | | Butyric acid | | Total | | Acetic acid | | Propionic acid | | Butyric acid | |
Location/Substrate	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Caecum														
Lactitol	29.8[a]	1.9	12.6[a]	0.9	5.0[a]	0.4	52.5[a]	2.4	63[b]	2	27[ab]	2	10[a]	1
Lactulose	45.1[c]	2.2	16.1[a]	0.9	11.9[b]	1.4	90.5[d]	2.9	62[b]	2	22[a]	1	16[ab]	2
OF	26.6[a]	1.9	12.7[a]	0.8	17.9[c]	1.8	67.2[bc]	2.6	47[a]	3	22[a]	1	31[c]	2
Mix OF-IN	31.1[ab]	2.0	16.0[a]	1.6	12.7[bc]	1.6	73.7[bc]	5.9	52[ab]	1	27[ab]	3	21[b]	2
IN	40.4[bc]	3.0	21.6[b]	1.6	11.1[b]	1.2	79.1[cd]	3.6	55[ab]	3	30[b]	1	15[ab]	2
IN-ls	31.3[a]	1.4	14.0[a]	0.8	7.8[ab]	0.4	59.7[ab]	2.3	59[b]	1	26[ab]	1	15[ab]	1
Proximal colon														
Lactitol	33.0[ab]	1.5	9.6[ab]	1.5	5.6[a]	0.8	65.7[a]	3.9	71[a]	3	18[ab]	2	11[a]	1
Lactulose	39.6[b]	3.6	7.9[ab]	1.2	5.4[ab]	1.0	83.2[a]	7.8	77[a]	2	14[a]	1	9[a]	1
OF	29.7[a]	2.0	6.1[a]	0.8	11.2[b]	2.2	65.6[a]	4.6	67[a]	3	12[a]	1	21[b]	3
Mix OF-IN	36.3[ab]	2.7	11.9[ab]	1.5	7.6[ab]	1.2	75.0[a]	3.9	66[a]	2	21[b]	1	13[a]	2
IN	33.4[ab]	3.4	12.8[b]	2.1	5.4[a]	0.6	66.6[a]	4.1	66[a]	2	23[b]	2	11[a]	1
IN-ls	32.7[ab]	1.7	10.0[ab]	1.3	9.0[ab]	1.7	61.9[a]	2.3	64[a]	2	19[ab]	2	17[ab]	2
Distal colon														
Lactitol	48.2[b]	2.7	11.9[a]	0.5	4.5[a]	0.5	86.3[ab]	5.1	74[b]		19[a]	1	7[a]	1
Lactulose	42.6[ab]	3.0	9.7[a]	1.4	8.3[ab]	2.2	84.8[ab]	3.0	72[b]	3	15[a]	1	13[a]	3
OF	39.9[ab]	1.7	12.6[a]	1.1	25.6[c]	3.1	99.5[b]	4.0	53[a]	3	16[a]	1	31[c]	2
Mix OF-IN	33.5[a]	3.9	10.1[a]	1.7	6.8[a]	1.2	69.6[a]	5.6	68[b]	2	19[a]	1	13[ab]	1
IN	43.0[ab]	4.6	25.5[b]	1.8	19.0[bc]	1.8	98.8[b]	5.4	48[a]	4	30[b]	2	22[b]	2
IN-ls	39.6[ab]	2.7	20.5[b]	1.8	17.0[bc]	2.3	88.4[ab]	6.3	52[a]	3	27[b]	1	21[b]	2

OF, Raftilose P95 (ORAFTI) fructo-oligosaccharides with a DP between 2 and 8. IN and IN-ls, Raftiline HP and Raftiline HPX both with an average DP of 23 (10–60), in which IN-ls has a lower solubility. Mix OF-IN, Raftilose SYNERGY1, a mixture of DP 2–8 (OF) and 10–60 (IN) (1:1).
[a,b,c,d]Mean values within a column and the same part of the hindgut with unlike superscripts are significantly different ($P<0.05$).

high proportions of acetic acid, throughout the hindgut. Comparatively high proportions of both butyric acid and propionic acid were found in the caecum of rats fed Mix OF-IN.

Caecal content and morphology. Feeding rats with diets containing indigestible carbohydrates increased the caecal wet content considerably compared with a control diet without any indigestible carbohydrates. The caecal tissue weight was related to caecal wet content ($R^2 = 0.81$), whereas no such relationship could be seen with caecal CA levels ($R^2 = 0.49$; Fig. 4).

Discussion

There is evidence that butyric and propionic acids may have health-promoting effects, and as the formation of SCFAs from carbohydrates in the colon appears to differ, it is of interest to study whether it is possible to alter SCFA formation by dietary means. In the present study, four types of fructo-oligosaccharide, lactulose and lactitol were used to study the potential effects of monomeric composition, DP and the solubility of the carbohydrates on SCFA formation, using a rat model. It can be
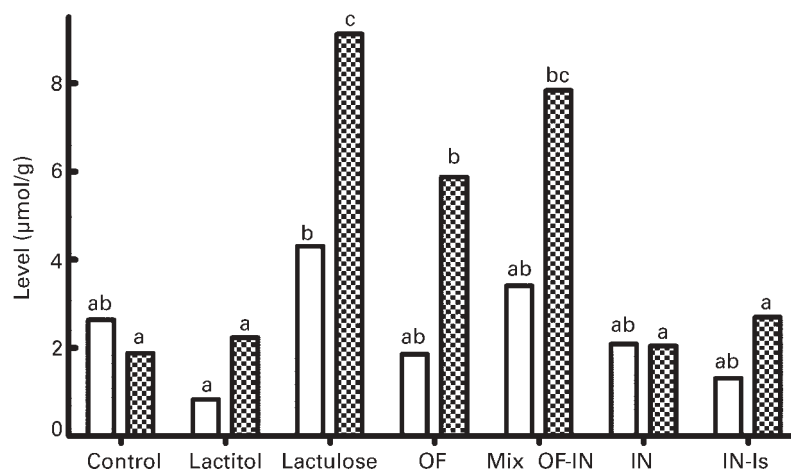
Fig. 2. Caecal levels (μmol/g wet content) of ($\square$) lactic and ($\boxplus$) succinic acid. Values with different superscripts between test diets for each acid are significantly different ($P<0.05$).
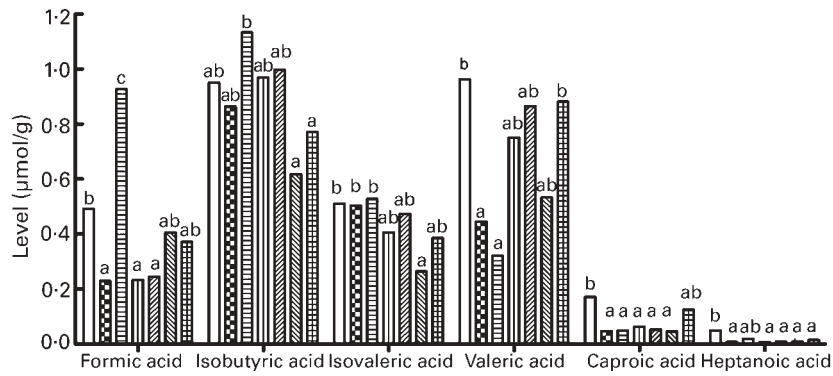
Fig. 3. Caecal levels (µmol/g wet content) of formic, isobutyric, isovaleric, valeric, caproic and heptanoic acids. (□) Control, (▦) lactitol, (▤) lactulose, (▥) oligofructose (OF), (▨) mixture of oligofructose and long-chain inulin (Mix OF-IN), (▧) long-chain inulin (IN), (⊞) long-chain inulin with low solubility (IN-ls). Values with different superscripts between test diets for each acid are significantly different ($P < 0.05$).

argued that the digestive tract of rats differs from that of man. However, it is difficult to study SCFA production in humans as the proximal colon, where most of the fermentation takes place, is inaccessible. Therefore, the rat is commonly used as an *in vivo* model. The rat model used in the present study has been shown to correlate well with studies in man with respect to total fermentability and bulking capacity (Nyman *et al.* 1986). Furthermore, *in vitro* fermentation has been shown to give similar SCFA profiles with faecal inocula from human subjects and rats (Lupton & Villalba, 1988; Barry *et al.* 1995), indicating that the rat is a relevant model for this purpose.

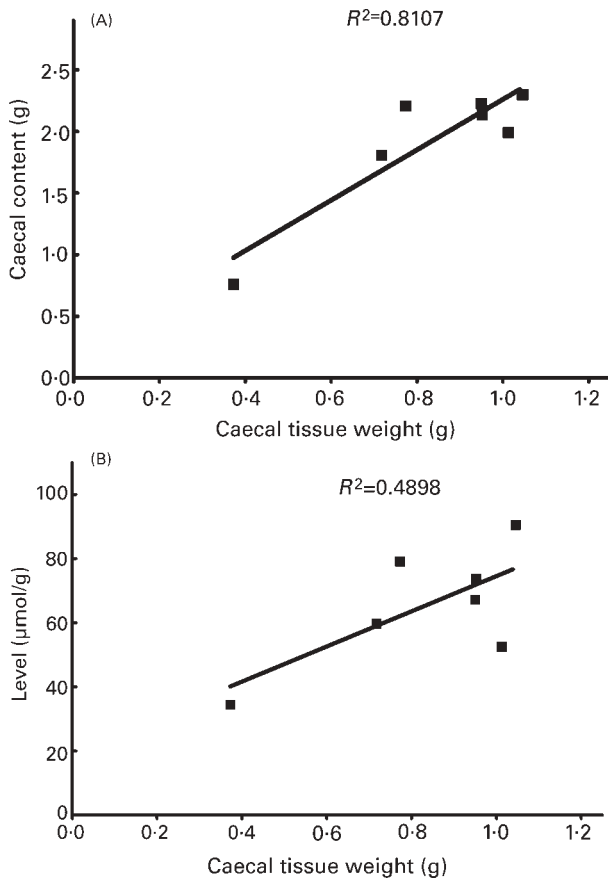**Fig. 4.** Correlation between caecal tissue weight and (A) caecal wet content, and (B) total levels of carboxylic acids in the caecum.

Feeding rats with diets containing oligosaccharides increased the caecal wet content considerably compared with a control diet without any indigestible carbohydrates. Interestingly, caecal tissue weight was correlated to a higher degree with caecal content than with caecal CA level ($R^2 = 0.81$ *v.* $R^2 = 0.49$), indicating that the hypertrophic effect observed in rats fed oligosaccharides was mainly due to increased caecal content rather than the formation of CAs. Similar observations have been made by others (Wyatt *et al.* 1988; Kishida *et al.* 2001) and explained by an increase in mechanical stress (Calvert *et al.* 1989). It has, however, also been suggested that the caecal enlargement is associated with an increase in mucosal cell proliferation, which is stimulated by SCFAs mediated by a systemic mechanism *in vivo* (Sakata, 1987). Furthermore, an increased production of SCFAs may increase proglucagon mRNA expression and the secretion of glucagon-like peptide-2, which are other factors affecting mucosal cell proliferation (Massimino *et al.* 1998). SCFA enemas have also been shown to have a transmural trophic effect and preserve the mucosal surface area of dysfunctional and atrophic colon in rats (Kissmeyer-Nielsen *et al.* 1995).

Diets containing IN and IN-ls led to higher faecal wet and dry weights than diets containing the other oligosaccharides. Both these carbohydrates had a higher DP than the other test materials. Interestingly, similar results have been found *in vitro*, where fructo-oligosaccharides with a DP greater than 10 were fermented much more slowly than fructo-oligosaccharides with a DP of less than 10 (Roberfroid *et al.* 1998). Furthermore, IN-ls, with a lower solubility than IN, was somewhat more resistant to hindgut fermentation, as judged by the higher faecal excretion of oligosaccharides with IN-ls than with IN. Feeding with IN-ls led to the lowest caecal pool of CAs (98 µmol *v.* 168 ± 8 µmol with the other fructo-oligosaccharides; Table 3), suggesting that IN-ls is fermented to a greater extent in the distal part of the hindgut. IN-ls was also the substrate that gave the highest caecal pH (6·9 *v.* a mean of 6·6 ± 0·1 for the other oligosaccharides; Table 2), providing further evidence of a higher resistance to caecal fermentation of this oligosaccharide than the others.

Another substrate that led to comparatively low caecal pools of CAs was lactitol, indicating a low degree of fermentation. However, feeding rats with this substrate gave the highest caecal content. An explanation of this could be that unfermented lactitol contributes to the osmosis in the caecum to a higher extent than SCFAs. Water would then be retained rather than absorbed

from the caecum, resulting in an enlarged, distended organ. Feeding rats with lactulose, on the other hand, resulted in the highest caecal levels and pools of CA, indicating that this disaccharide is rapidly fermented. Interestingly, in studies on humans lactulose resulted in more pronounced changes in, for example, SCFA production and the activity of pro-carcinogenic enzymes compared with lactitol (Ballongue *et al.* 1997), as well as to a lower colonic pH (5·1 *v.* 5·6 with lactitol; Patil *et al.* 1987).

The effects on microbial composition also seem to be different between these two disaccharides. Thus, lactulose has been shown to selectively stimulate the number of bifidobacteria in man (Tuohy *et al.* 2002), whereas lactitol decreased the bifidobacterial and *Bacteroides* populations in an *in vitro* culture system when added to a growth medium containing a low amount of dietary fibre (Probert *et al.* 2004). In the same *in vitro* model, oligofructose was shown to increase the number of bifidobacteria, bacteria that have been proven to have a probiotic potential in man (Gibson & Wang, 1994). However, contrary to the present study, lactitol was more prone to producing butyric acid than oligofructose (Probert *et al.* 2004).

Lactulose generally had low levels and proportions of propionic acid, and high levels and proportions of acetic acid (Table 4). This may have metabolic consequences, as propionic acid has been suggested to lower serum lipid and cholesterol levels. Thus, when subjects were given rectal infusions of propionate and acetate, it was shown that propionate repressed the utilisation of acetate for the synthesis of cholesterol (Wolever *et al.* 1991). Similar results were obtained in obese rats (Berggren *et al.* 1996). Interestingly, in a human study on healthy subjects, lactulose was shown to increase serum lipids and LDL-cholesterol (Jenkins *et al.* 1991), and the speculation is whether this was due to the high amounts of acetic acid formed. In a study on non-insulin-dependent diabetic patients, serum total and LDL-cholesterol were lowered by fructo-oligosaccharides (Yamashita *et al.* 1984). The discrepancies in result between fructo-oligosaccharides and lactulose were suggested to be due to a higher propionate acetate: ratio with fructo-oligosaccharides than with lactulose (Luo *et al.* 1996). Another explanation could be differences in metabolic status between healthy and non-insulin-dependent subjects.

Rats fed diets containing OF had the highest caecal pools and levels of butyric acid, whereas rats fed diets containing IN had the highest caecal pools (together with Mix OF-IN) and levels of propionic acid. As both of these carbohydrates are composed of fructose with β-2,1 linkages it seems that other factors, such as DP, are of importance. OF consists of fructo-oligosaccharides with a DP between 2 and 8, whereas IN contains fructo-oligosaccharides with a DP of between 10 and 60. In most studies, fructo-oligosaccharides have been found to give high yields of butyric acid, at least compared with other oligosaccharides (Roland *et al.* 1995; Campbell *et al.* 1997; Poulsen *et al.* 2002). However, in one study employing different doses of inulin (fructo-oligosaccharides with a higher DP), high proportions of propionic acid were formed (Levrat *et al.* 1991). High amounts of propionic acid with IN were also obtained in rats fed fibre diets partially replaced by fructo-oligosaccharides (IN or oligofrucotse; Kleessen *et al.* 2001). However, the levels of butyric acid were in the same range as the two fructo-oligosaccharides, which was higher than with the control diet in that study. This could be explained by the fact that the diet contained other indigestible carbohydrates. It has thus been shown that certain combinations of indigestible carbohydrates yielded a higher proportion of butyric acid than

did the single substrates (Henningsson *et al.* 2002). Rats fed a diet containing OF also gained most in weight. Fermentable carbohydrates are known to provide energy to the host (6·3–7·1 kJ/g) (Livesey *et al.* 1995; Roberfroid, 1999). However, in this study all carbohydrates tested are easily fermented. It can therefore be questioned whether the SCFA profile could be of importance in this context.

The butyric acid level increased along the hindgut of rats fed OF, IN and IN-ls. Similar results were observed for propionic acid in rats fed IN and IN-ls. The transit time through the gastrointestinal tract has been reported to influence bacterial activities and pathways and, as a result, the proportion of individual SCFAs may be changed (Oufir *et al.* 2000). It has been reported that the shorter the caecal transit time, the higher the proportion of butyric acid (Mathers & Dawson, 1991). As the transit time was not measured in the present study, it remains to be shown whether this is also the case for OF, IN and IN-ls. The increased level of butyric acid along the hindgut in rats fed IN-ls may also be due to the slower fermentation of this substrate. Wheat bran, another substrate that is slowly fermented, has been shown to lead to large amounts of butyric acid in the distal part of the hindgut (Henningsson *et al.* 2002). This is interesting and might be important from a nutritional point of view, as most colonic diseases also occur in the distal part of colon.

We conclude that the DP and solubility of the fructo-oligosaccharides are of great importance for the SCFA formation. Oligosaccharides with a low DP (OF) generated a high level of butyric acid, whereas those with a high DP (IN) gave a high level of propionic acid. A lower solubility of the fructo-oligosaccharides was related to a lower degree of caecal fermentation and a higher formation of butyric acid in the distal part of colon. This means that different types of fructo-oligosaccharides can be used for different health effects when designing foods. The fructose content *per se* seems to be of less importance as lactulose, containing galactose and fructose, and lactitol (galactose and glucitol) both yielded high proportions of acetic acid and low proportions of butyric acid.

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