Original Article

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A water-soluble fraction from a by-product of wheat increases the formation of propionic acid in rats compared with diets based on other by-product fractions and oligofructose

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Abstract

Background: Dietary fibre is fermented by the colonic microbiota to carboxylic acids (CA), with potential health effects associated in particular with butyric and propionic acid.
Objective: To investigate the formation of CA in the hindgut of healthy rats fed dietary fibre from different fractions of wheat shorts, a by-product of the milling of wheat.
Design: Rats were fed dietary fibre (80 g/kg feed per day for 7 days) from wheat shorts and fractions thereof (ethanol-soluble, water-soluble and insoluble fractions), oligofructose (OF) diet and a mixture of oligofructose and raffinose (OR) diet.
Results: The water-soluble fraction, with a high content of arabinoxylan (AX), increased the formation of propionic acid in the hindgut and lowered the ratio of acetic to propionic acid in the portal blood of rats. High levels and proportions of butyric acid were seen in rats fed the OR diet. The pattern of CA resulting from the ethanol-soluble diet, mainly composed of fructan and raffinose, was more similar to that of the OF diet than the OR diet.
Conclusions: The high formation of propionic acid with the water-soluble fraction may be attributed to the high AX content. The results also indicate that the wheat fructans produced more propionic acid and less butyric acid than oligofructose. It may furthermore be speculated that the increased formation of butyrate with the OR diet was due to synergistic effects of the components in this diet.

Keywords: *dietary fibre*; *arabinoxylan*; *fructan*; *short-chain fatty acids*; *carboxylic acids*

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Dietary fibre is not digested in the small intestine, but reaches the large intestine, where it is fermented by the microbiota to short-chain fatty acids (SCFA) and other carboxylic acids (CA) and gases. The principal acids formed are acetic, propionic and butyric acids, of which propionic and butyric acids show the most interesting health-promoting effects. Propionic acid has been proposed to exert a lipid-lowering effect through inhibition of the production of cholesterol from acetic acid. Furthermore, propionic acid may influence the glucose metabolism in two opposing ways, both as a substrate and as an inhibitor for gluconeogenesis (1). Butyric acid is the primary energy source for the colonocytes but has also been suggested to strengthen the colonic defence, providing protection against colon cancer (2). A recent study further indicates that butyric acid may be associated with improved glucose tolerance (3). The formation of CA also results in a decrease in pH, which may lead to a reduction in the growth of pathogenic bacteria and an increase in mineral absorption (1). In addition, dietary fibre may selectively stimulate bacteria such as bifidobacteria and lactobacilli, which are associated with health benefits (4). The monosaccharide composition seems in part to be of importance for the relative proportions of SCFA formed, but other characteristics of the dietary fibre,

such as the type of linkages between monosaccharides, the solubility of the dietary fibre polysaccharides and the combination of substrates, have also been proposed to have an effect (5, 6).

Wheat shorts, a by-product of the milling industry, contain fine particles of bran, endosperm and part of the germ, and is often used for animal feed (7, 8). Arabinoxylan (AX) and fructan are two dietary fibre components with potential health-promoting effects that are found in this fraction. Recently, several studies have focused on the effects of AX and arabinoxylan oligosaccharides (AXOS) on fermentation in the large intestine (9–11). The physiological properties of commercially available inulin-type fructans with (1→2)-linked β-D-fructofuranose units have been well characterised, in contrast to the (1→2)- and (6→2)-linked graminan-type fructans in wheat. Wheat shorts have been reported to contain about 3–4% fructan (dry weight basis, dwb) with a degree of polymerisation (DP) of up to 19 (12).

In this study, the formation of CA in the hindgut of healthy rats fed dietary fibre from shorts was investigated. Shorts were fractionated into an ethanol-soluble, a water-soluble and an insoluble part, in an attempt to separate fructans, soluble AX and insoluble AX into different fractions. Rat diets were prepared from these new fractions and from the original shorts fraction, and their effects compared with a diet containing commercially available oligofructose. An additional diet was prepared with the dietary fibre composed of oligofructose and raffinose in the same proportion as in the ethanol-soluble diet, in an attempt to compare the effects of wheat fructans with those of inulin-type fructans.

Materials and methods

Dietary fibre fractions

Shorts from wheat flour (*Triticum aestivum*, cultivar Harnesk) from Lantmännen (Lidköping, Sweden) were fractionated into an ethanol-soluble, a water-soluble and an insoluble part (Fig. 1). Batches of 250 g shorts were extracted with 1,000 ml 70% (v/v) ethanol at 50°C for 1 hour with occasional stirring, and then filtered through a Büchner funnel. The filter cake was washed with an additional volume of 250 ml ethanol and was left for 10 min before filtering. The ethanol fraction was concentrated in a vacuum centrifuge and the final ethanol evaporated in a rotary evaporator. Wheat starch (Cerestar, Krefeld, Germany) was added at a level of about 0.5 g starch per g ethanol-soluble fraction to facilitate freeze-drying. The mixture was freeze-dried and mortared to a powder. Batches of 130 g air-dried filter cake were mixed with 500 g water and 100 mg endo-xylanase (Xylanase BS 80 K; Danisco, Grindsted, Denmark) for 2 min in a household blender, and then incubated at 40°C for 2 hours. The xylanase was added to improve the yield of water-soluble AX. The slurry was centrifuged (1,000 *g*, 10 min) and the pellet washed by mixing with 500 ml water for 2 min in the blender before being centrifuged again. The supernatants from both centrifugations (the water-soluble fraction) and the pellet (the insoluble fraction) were freeze-dried and ground to a powder in an ultracentrifugal mill with a 0.5 mm ring sieve (Retsch, Haan, Germany).

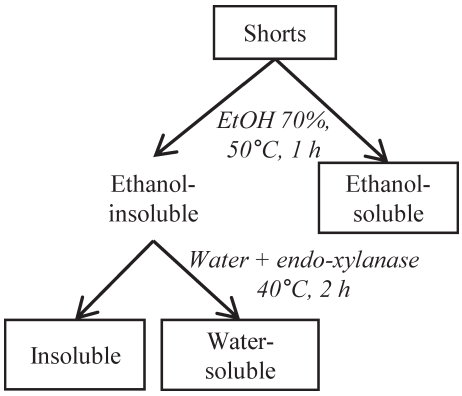

Fig. 1. Fractionation of shorts into ethanol-soluble, water-soluble and insoluble fractions.

Preparation of diets

One test diet was prepared for each of the four dietary fibre fractions from shorts. Two diets were prepared with oligofructose (Beneo®P95; ORAFTI, Tienen, Belgium), which is composed of inulin-type fructans with a DP of 2–8, and was considered to be the commercially available material with the most similar DP range to the wheat fructans. One of these diets contained only OF (the OF diet) and the other diet consisted of a mixture of oligofructose and raffinose (Merck, Darmstadt, Germany) at the same ratio as wheat fructans to raffinose in the ethanol-soluble diet (hereafter referred to as the OR diet). Each diet contained casein (Sigma Chemical Company, St Louis, MO, USA), sucrose (Danisco Sugar, Malmö, Sweden), maize oil, DL-methionine (Sigma Chemical Company), choline chloride (Aldrich Chemie, Steinheim, Germany), a vitamin mixture and a mineral mixture (both from Lantmännen, Lidköping, Sweden). The fibre fractions were added at a level of 80 g dietary fibre/kilogram feed. Wheat starch (Cerestar Deutschland GmbH, Krefeld, Germany) was used to adjust the dry matter content (see Table 1). This starch product has been shown to be completely digested and therefore does not contribute to the formation of CA.

Experimental design

Forty-two male Wistar rats (Scanbur, Sollentuna, Sweden), about 4 weeks old, weighing 98±4.9 g (standard deviation [SD]) were divided into six groups of seven animals. Each group was assigned one of the diets,

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Table 1. Composition of the test diets

Component	Amount (g/kg dry weight)
Dietary fibre fraction[a]	83–313
Casein	120
dl-methionine	1.2
Maize oil	50
Mineral mixture[b]	48
Vitamin mixture[c]	8
Choline chloride	2
Sucrose	100
Wheat starch[a]	358–588

[a]The sum of the fibre fraction and starch was constant; the proportions being adjusted to obtain a concentration of 80 g dietary fibre per kg feed (dwb).

[b]Containing (g/kg) 0.37 $CuSO_4 \cdot 5H_2O$, 1.4 $ZnSO_4 \cdot 7H_2O$, 332.1 KH_2PO_4, 171.8 $NaH_2PO_4 \cdot 2H_2O$, 324.4 $CaCO_3$, 0.068 KI, 57.2 $MgSO_4$, 7.7 $FeSO_4 \cdot 7H_2O$, 3.4 $MnSO_4 \cdot H_2O$, 0.020 $CoCl \cdot 6H_2O$ and 101.7 NaCl.

[c]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 and 941.25 maize starch.

and the rats were fed 12 g dry matter/day and had unlimited access to water. The rats were housed individually in metabolic cages and allowed to adapt to the diet for 7 days before the experimental period of 5 days. Weight gain and food consumption were measured during the experimental period. Faecal samples were collected daily and stored at −20°C.

After the experimental period the animals were anaesthetised by subcutaneous injection of a mixture of Hypnorm® (Division of Janssen-Cilag Ltd, Janssen Pharmaceutica, Beerse, Belgium), Dormicum® (F. Hoffman-La Roche AG, Basel, Switzerland) and sterile water (1:1:2) at a dose of 1.5 ml/kg body weight. Blood was taken from the hepatic portal vein for the analysis of SCFA, placed in serum tubes, centrifuged and transferred to vials for storage at −40°C. The caecum and proximal and distal colon were removed. The caecum was weighed immediately, the content was transferred to test tubes and pH was measured. The caecal content, proximal colon and distal colon were stored at −40°C for later analysis of CA. Caecal tissue weight was also determined. The Malmö/Lund Ethical Committee on Animal Experiments approved the animal experiments.

Composition of diets

The total carbohydrates in the diets were determined using gas-liquid chromatography for neutral sugars, spectrophotometry for uronic acids and gravimetrical determination of Klason lignin (13) with the following modifications. Starch degradation with Termamyl® and amyloglucosidase was omitted. Samples were extracted with 20 ml water (40°C, 15 min), centrifuged at 1,000 *g* and the supernatants removed. The pellets were freeze-dried and incubated with 12 M sulphuric acid before being combined with their respective supernatants to prevent low-molecular-weight material from being degraded by the acid. The original method was followed from the autoclaving step. Furthermore, Klason lignin was determined according to the original method for the shorts and the insoluble fraction to remove ethanol-soluble components, which could otherwise be falsely included in the Klason lignin. The ethanol-soluble and water-soluble fractions were assumed to contain no Klason lignin, which was confirmed by analysis of one batch of the water-soluble fraction according to the original dietary fibre method (data not shown). The fructan content was determined with the enzymatic/spectrophotometric AOAC method, 999.03 (14), as described previously (12). Starch, including maltooligosaccharides and glucose, was analysed using the method described by Holm et al. (15). Glucose, fructose, sucrose and raffinose contents were analysed by Eurofins Food & Agro Sweden AB (Lidköping, Sweden). Digestible carbohydrates were calculated as the sum of starch (including maltooligosaccharides), glucose, fructose and sucrose. Dietary fibre was calculated as the sum of fructan, raffinose, neutral sugar residues, uronic acid residues and Klason lignin. Neutral sugar residues were corrected for glucose derived from starch, glucose, fructans [approximating the average DP to 6 (12)] raffinose and sucrose, and for galactose from raffinose. The contents of protein (Kjeldahl, $N \times 5.83$), crude fat [Schmid-Bondzynski-Ratzlaff (16)] and ash were analysed by Eurofins Food & Agro Sweden AB (Lidköping, Sweden). The AX and arabinogalactan (AG) contents were calculated from arabinose, xylose and galactose residues, as suggested by Delcour et al. (17), assuming arabinose to be included in AG with an Ara/Gal ratio of 0.7. The composition of the original ethanol-soluble fraction was obtained by correcting for the addition of starch before analysis. The amount of the ethanol-soluble fraction added to the diet was based on the analysed composition of the ethanol-soluble fraction mixed with starch.

Analysis of the molecular weight distribution of fructan

The molecular weight distribution of fructan was determined using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), as described by Rakha et al. (18). The analysis included the treatment of samples with inulinase for the identification of fructan peaks.

Fermentability

Faecal samples were freeze-dried and milled. The composition of remaining carbohydrates (neutral sugars and uronic acids) was determined with the method of Theander et al. (13) but without the dietary fibre precipitation in ethanol and without analysis of the Klason lignin. Fructan and raffinose were assumed to be completely degraded by the microbiota (5, 19), and no analyses were performed on faeces from rats who had consumed mainly these dietary fibre components, i.e. rats fed the ethanol-soluble, OF and OR diets.

Carboxylic acids

Gas-liquid chromatography was used to determine the SCFA in the caecum and colon (20) and in portal blood (21), with an HP 6890 gas chromatograph (Hewlett Packard, Wilmington, DE, USA), with an HPDB-FFAP 125-3237 column (J&W Scientific, Agilent Technologies Inc., Folsom, CA, USA). The method for analysis in portal blood was modified to include the addition of an internal standard (2-ethylbutyric acid) before analysis. The internal standard was used for quantification in the same way as in the method for the caecum and colon. The amounts of lactic and succinic acid in the caecum were determined by ion-exclusion chromatography with suppressed conductivity detection using a MIC-12 EDU IC system (Metrohm AG, Herisau, Switzerland) consisting of a high-pressure 818 IC pump unit, an 820 IC separation centre with a column oven and a suppressor module connected to an 833 IC liquid handling unit, an 819 IC conductivity detector and an 830 IC interface for connection to a PC. Chromatograms were evaluated with Metrohm IC Net 2.3 software. Samples were taken from the caecal content that had been prepared for the gas chromatographic analysis and were further diluted 1:10 with Millipore water, filtered through a 0.45 syringe filter and injected via a 20 µl loop onto a Metrosep organic acids column (250 × 7.8 mm, Metrohm) mounted in a column oven set at 40°C. The elution solvent was 0.5 mM sulphuric acid with 10% acetone, at a flow rate of 0.5 ml/min. The run time for each analysis was 25 min. The suppressor was regenerated with a solution of 10 mM LiCl followed by water. The levels of lactate and succinate in the samples were calculated using calibration curves obtained at four concentrations (5, 10, 25 and 100 µM) on three different occasions. The yield was approximated as the yield of internal standard in the gas chromatographic analysis.

Calculations and statistical analysis

Caecal pools of CA were calculated as the concentration of each acid multiplied by the weight of the caecal content. The results were extrapolated to complete intake of the food given. Heptanoic acid was present at levels close to the detection limit of about 0.05 µmol/g. When the acid was not detected, a concentration of 0 µmol/g was used to calculate mean values. One-way ANOVA with Tukey's test was used to evaluate the effect of the different diets. Differences were considered significant at $P < 0.05$. Differences between levels of CA in the caecum and distal colon were analysed with the paired t-test ($P < 0.05$). Before statistical evaluation, caecal pools and the levels and proportions of acetic, propionic and butyric acid were transformed with the Box–Cox transformation when not normally distributed, according to the Anderson–Darling normality test. All statistical tests were performed with Minitab statistical software (Release 16).

Results

Composition of shorts fractions

The highest dietary fibre content was found in the ethanol-soluble fraction (620 g/kg dwb) followed by the water-soluble fraction (527 g/kg dwb) (Table 2). The insoluble fraction and shorts contained similar amounts of dietary fibre (376 and 338 g/kg dwb, respectively). The sum of the analysed components was higher for the ethanol-soluble fraction (1312 g/kg dwb, corrected for the addition of starch before freeze-drying) than for the other diets (958–1,019 g/kg dwb).

The quantitatively most important dietary fibre components in the shorts were AX (43.3%), fructan (17.6%), glucose-containing polysaccharides (13.7%) and Klason lignin (10.3%). The dietary fibre from the insoluble fraction contained the highest proportion of glucose-containing polysaccharides (34.4%) while the content of AX and Klason lignin was comparable to that of the original shorts fraction. The dietary fibre from the water-soluble fraction was enriched in water-soluble AX (58.9%) but also contained about the same proportion of fructan and glucose-containing polysaccharides as the shorts. The dietary fibre that was part of the ethanol-soluble fraction was composed of low-molecular-weight carbohydrates (67.4% fructan and 15.2% raffinose). The Ara/Xyl ratio was 0.58 in the shorts, 0.43 in the water-soluble fraction and 0.54 in the insoluble fraction.

The fructan obtained in the ethanol-soluble fraction had a molecular size distribution similar to that of the fructan in the shorts fraction, indicating a representative extraction yield (Fig. 2). The water-soluble fraction also contained fructan with a similar size distribution, but the anion-exchange chromatogram also revealed many other oligosaccharides that are likely to originate from AX. The relative distribution of the DP of fructan (DP 3–4:DP 5–9:DP 10–15:DP >16) was 31:36:29:3 for the shorts, 25:38:34:3 for the water-soluble fraction and 27:36:34:3 for the ethanol-soluble fraction.

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Table 2. Composition of the fibre fractions (g/kg)

	Shorts	Insoluble	Water-soluble	Ethanol-soluble	OF[a]	OR[a]
Dietary fibre	338	376	527	620	957	965
Fructan (%)	17.6	2.0	16.8	67.4	100	81.6
Raffinose (%)	3.9	ND	2.6	15.2	–	18.4
Arabinose (%)	17.1	16.5	20.2	1.4	–	–
Xylose (%)	28.1	25.5	41.3	0.8	–	–
Mannose (%)	1.6	1.7	0.4	1.0	–	–
Galactose (%)	2.7	2.5	3.7	2.6	–	–
Glucose (%)	13.7	34.4	13.2	8.6	–	–
Klason lignin (%)	10.3	11.7	–	–	–	–
Uronic acids (%)	4.9	5.7	1.8	3.1	–	–
AX (%)[b]	43.3	40.2	58.9	0.8[c]	–	–
AG (%)[b]	4.7	4.2	6.2	4.0[c]	–	–
Ara/Xyl[b]	0.54	0.58	0.43	–	–	–
Digestible carbohydrates	368	431	80	224	43	35
Starch (%)[d]	93.5	99.8	62.8	0.0	–	–
Glucose (%)	0.4	0.2	9.3	2.7	–	–
Fructose (%)	0.2	ND	5.2	1.9	–	–
Sucrose (%)	5.9	ND	22.7	95.5	–	–
Crude fat	69	53	128	151	–	–
Protein	144	133	150	273	–	–
Ash	39	26	105	44	–	–
Total	958	1,019	990	1,312	1,000	1,000

OF, oligofructose; OR, oligofructose and raffinose; ND, not detected at a detection limit of 0.4 g/kg.

[a]According to manufacturers' specifications.

[b]AX = % Ara 0.7 × % Gal + % Xyl, AG = (1.7 × % Gal), Ara/Xyl = (% Ara 0.7 × % Gal)/% Xyl (17).

[c]Data uncertain because too little arabinose was present to calculate AG according to the formula, and the value for AX thus only contains xylose.

[d]Including maltooligosaccharides and maltose.

Feed intake and weight gain, and caecal content, tissue weight and pH

The feed intake was almost complete by the rats fed the shorts, insoluble fraction, OF and OR diets, with an average intake of 11.8–11.9 g/day; the lowest individual intake being 11.3 g/day (Table 3). In contrast, a larger variation was seen among the rats fed the ethanol-soluble diet (mean 11.1, range 9.1–12.0 g/day) and the water-soluble diet (mean 10.7, range 9.4–12.0). The diet containing the insoluble fraction led to a lower caecal content (1.9 g) in the rats than those fed the soluble diet and the OF and OR diets (3.9 and 3.5 g, respectively), while the caecal content of the rats fed the shorts diet (2.1 g) was only lower than that in rats fed the OF diet. Gas was found in the caecum of several rats in the OR group. According to visual observation, the caecal content and faeces were more liquid in the rats fed the OR and OF diets than in those fed the shorts diet. Caecal tissue weight was highest in the OR group (0.94 g) and was higher in the OF group (0.74 g) than in all the groups fed the wheat shorts fractions, except for the water-soluble fraction (0.65 g). When the caecal tissue weight is plotted against the caecal content, a different pattern can be seen in the OR group compared with the other groups (Fig. 3). The positive correlation when only looking at the other groups was significant ($R^2 = 42.6\%$, $P < 0.001$). The weight gain (g/g feed) and caecal pH did not differ between the groups. Total caecal pools were negatively correlated with caecal pH ($P < 0.001$, $R^2 = 52\%$).

Fermentability

Results regarding the faecal excretion of dietary fibre, based on the analysis of neutral sugar residues using gas chromatography (GC) and uronic acids, showed that 3% of the dietary fibre in the water-soluble fraction, 50% of that in the shorts and 44% of the dietary fibre in the insoluble fraction were excreted (Table 4). Assuming complete fermentation of raffinose and fructans would change the dietary fibre excretion to 38% for the shorts and 43% for the insoluble fraction. The AX in the water-soluble fraction was almost completely fermented, with only 1% of the arabinose and xylose appearing in the faeces. In contrast, the arabinose and xylose of the insoluble fraction was much more resistant to fermentation, and 62 and 40% were excreted in faeces, respectively.

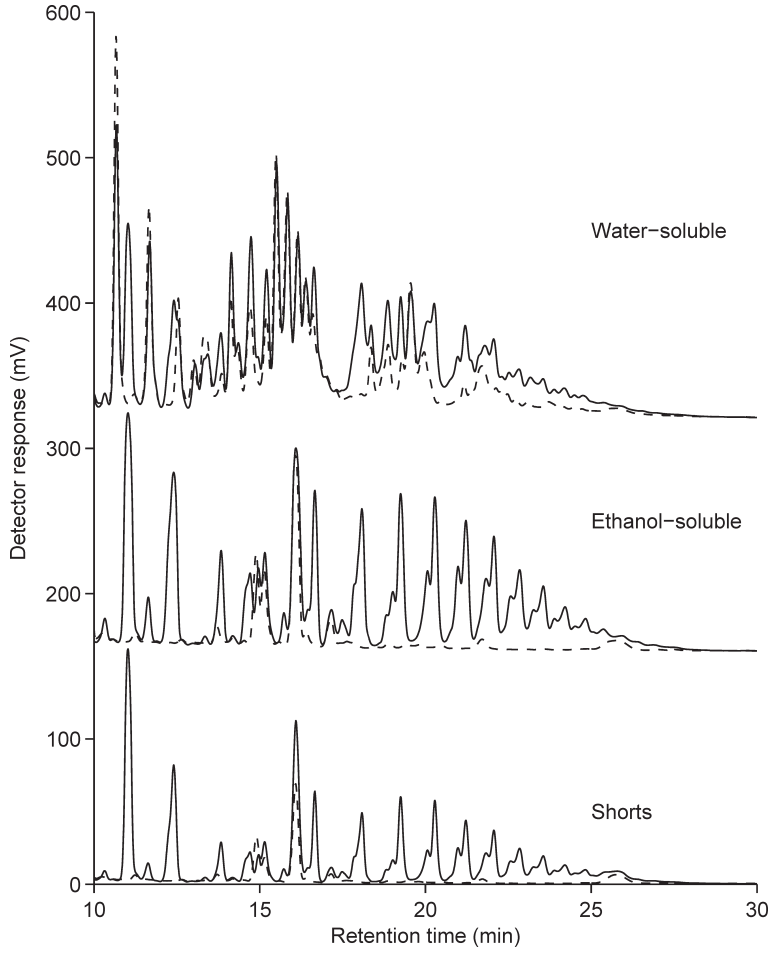

Fig. 2. HPAEC-PAD chromatograms for the shorts, the water-soluble and the ethanol-soluble fractions. A dashed line is used for samples treated with inulinase.

The glucose-containing polysaccharides in the insoluble fraction were less resistant to fermentation than the glucose-containing polysaccharides from shorts (38 vs. 86% were excreted in faeces).

Carboxylic acids in the caecum and colon

The caecal pool of propionic acid was higher in rats fed the water-soluble diet (73 μmol) than in those fed all the other diets, but it was not significantly different from that in rats fed the ethanol-soluble diet. The ethanol-soluble fraction (45 μmol) led to a larger caecal pool of propionic acid in the rats than did the shorts, the insoluble fraction and the OR diet (17–29 μmol) (Table 5). Furthermore, this fraction together with shorts led to more caecal isobutyric acid than the water-soluble and OR diets, and also more isovaleric acid than the

Table 3. Feed intake, weight gain, caecal content, caecal tissue weight and caecal pH

	Shorts	Insoluble	Water-soluble	Ethanol-soluble	OF	OR
Feed intake (g/day)	11.9 ± 0.0a	11.9 ± 0.1a	10.7 ± 0.5b	11.1 ± 0.4ab	11.8 ± 0.1a	11.8 ± 0.0ab
Weight gain (g/g feed)	0.33 ± 0.01	0.30 ± 0.01	0.30 ± 0.06	0.36 ± 0.02	0.30 ± 0.03	0.29 ± 0.03
Caecal content (g)	2.1 ± 0.2bc	1.9 ± 0.4c	3.8 ± 0.4a	3.3 ± 0.4abc	3.9 ± 0.5a	3.5 ± 0.5ab
Caecal tissue (g)	0.50 ± 0.01cd	0.44 ± 0.01d	0.65 ± 0.04bc	0.48 ± 0.03d	0.74 ± 0.04b	0.94 ± 0.06a
Caecal pH	6.9 ± 0.1	7.0 ± 0.1	6.5 ± 0.1	6.7 ± 0.1	6.8 ± 0.1	6.6 ± 0.2

Note: All feed weights are given on a dry weight basis. Results are given as means ± SEM, $n = 6$ for OR and $n = 7$ for all other groups. Means that do not share a letter are significantly different ($P < 0.05$).
OF, oligofructose; OR, oligofructose and raffinose.

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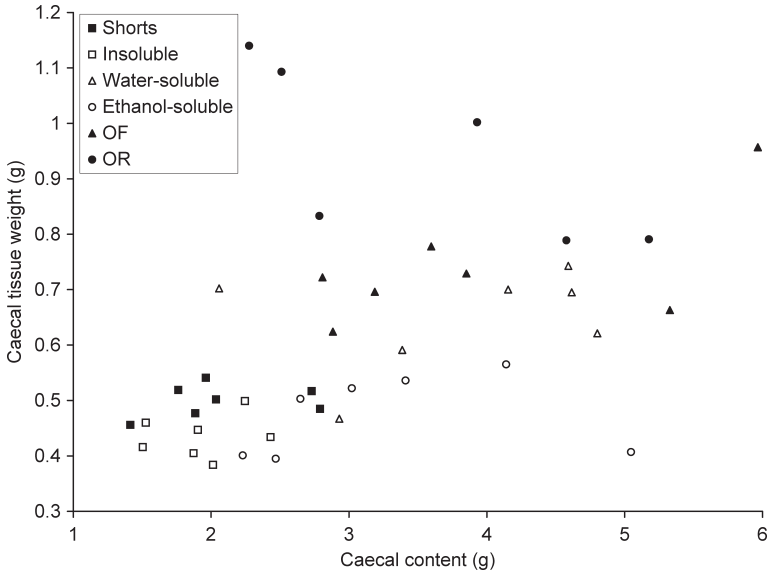

Fig. 3. Correlation between caecal tissue weight and caecal content.

OR diet. Caecal pools of lactic acid were higher in rats fed the OR and ethanol-soluble diets than in rats fed the insoluble fraction, while caecal pools of succinic acid were higher in rats fed the OF, OR and water-soluble diets than in rats fed the shorts and insoluble diets. The caecal pool of valeric acid was higher in the rats fed the shorts and insoluble diets than in groups fed the OF and OR diets.

Two rats exhibited very different values of SCFA in the caecum and colon compared with the other rats in the same group; to such an extent that the Box–Cox transformation was insufficient to make all the data normally distributed. One of these rats, with particularly high levels of butyric acid (ratio of acetic:propionic: butyric acid 37:18:45), belonged to the OF group, and the other rat, with low levels of propionic and butyric acid (ratio of acetic:propionic:butyric acid 82:7:11), belonged to the OR group. Therefore, data from these rats were excluded from all calculations of levels and proportions (Table 6).

Table 4. Faecal excretion of dietary fibre (Percentage of ingested dietary fibre)

	Shorts	Insoluble	Water-soluble
Fructan	–	–	–
Raffinose	–	–	–
Arabinose	52±1	62±2	1±0
Xylose	33±1	40±1	1±0
Mannose	30±2	30±2	32±2
Galactose	49±2	57±2	19±1
Glucose	86±3	38±2	7±0
Klason lignin	–	–	–
Uronic acids	43±1	43±1	16±1
Total	50±2	44±2	3±0

Note: Results are given as means±SEM, $n=7$.

The OR diet led to the highest proportion of butyric acid throughout the hindgut (27–32%) and a higher level in the distal colon than with the ethanol-soluble and OF diets. The proportion of propionic acid was highest in rats fed the water-soluble diet in the caecum (30%) and proximal colon (28%), and higher than in rats fed the shorts and insoluble diets also in the distal colon (23%). The proportions of acetic acid were lower in rats fed the water-soluble diet (59%) and the OR diet (57%) in the caecum than in the rats fed the other diets (66–69%), with differences becoming less significant along the colon. When the two outliers were included in the calculations, no significant differences were found for acetic acid, and the proportions of butyric acid were only different in the caecum; a higher proportion being found in the OR group than the water-soluble and ethanol-soluble groups. The levels of butyric acid were lower in the distal colon than in the caecum of rats fed the shorts and ethanol-soluble diets, while the differences in the other groups were not significant (data not shown). The acids present in minor amounts (isobutyric, isovaleric, valeric, caproic and heptanoic acids) were generally found at higher levels and proportions (calculated as percent of the total concentration of SFCA) in rats fed the diets containing shorts and the insoluble fraction, and were lower in rats fed the water-soluble fraction, OF and OR diets (data not shown).

Short-chain fatty acids in portal blood

The levels of acetic acid and total SCFA in portal blood were higher in rats fed the shorts diet (1193 and 1409 μmol/g, respectively) than in rats fed the insoluble

Table 5. Caecal pools (μmol) of carboxylic acids

	Shorts	Insoluble	Water-soluble	Ethanol-soluble	OF	OR
Acetic	109±11	80±4.6	135±24	143±16	99±17	102±31
Propionic	20±2.8c	17±1.0c	73±15a	45±5.8ab	29±3.0bc	22±5.6c
Isobutyric	2.4±0.4a	1.8±0.2ab	1.1±0.1bc	2.6±0.3a	1.6±0.4abc	0.7±0.2c
Butyric	29±3.9	18±1.0	27±5.4	28±3.0	31±7.8	39±12
Isovaleric	3.1±0.5ab	2.5±0.2ab	2.0±0.3ab	3.8±0.5a	3.2±0.6ab	1.5±0.3b
Valeric	2.5±0.3a	2.0±0.1ab	0.9±0.2bc	2.1±0.8abc	0.7±0.1c	0.6±0.1c
Caproic	1.1±0.4ab	0.9±0.2a	0.2±0.0b	0.9±0.5ab	0.3±0.1ab	0.1±0.0b
Heptanoic[a]	0.1±0.0 (3)	0.1±0.0	0.0±0.0 (7)	0.1±0.1 (3)	0.0±0.0 (6)	0.0±0.0 (7)
Lactic	4.4±1.2ab	2.7±0.6b	7.2±2.3ab	9.9±3.3a	5.5±1.1ab	17±5.3a
Succinic	2.0±0.3b	1.7±0.4b	8.2±1.2a	4.5±0.9ab	10±4.3a	9.2±2.5a
Total	173±19	127±6.3	255±44	240±24	180±24	192±52

Note: Results are given as means±SEM, $n=6$–7. Means that do not share a letter are significantly different ($P<0.05$).
OF, oligofructose; OR, oligofructose and raffinose.
[a]The numbers in parentheses indicate the number of rats in the group for which the acid was not detected at a detection limit of about 0.05 μmol/g.

diet (872 and 1016 μmol/g, respectively) (Table 7). The water-soluble diet resulted in more propionic acid (163 μmol/g) than the OR diet (65 μmol/g), but the difference disappeared upon the removal of one particularly high value (419 μmol/g) from the group given the water-soluble fraction. The ratio of acetic to propionic acid was lower in rats fed the water-soluble diet (7.6) than in rats fed the shorts, insoluble, OF and OR diets (13–18). The difference for the OR diet disappeared when one rat with a very high value (40 μmol/g) was removed. The shorts diet led to more isobutyric and isovaleric acids (13 and 15 μmol/g, respectively) in the portal blood of rats than the water-soluble (6.5 and 7.0 μmol/g, respectively) and OR (5.2 and 6.4 μmol/g, respectively) diets. Levels of all acids analysed in portal blood were correlated with caecal pools ($P<0.005$, $R^2=31.5$–56.1%).

Discussion

Shorts is a by-product of milling that is often used for animal feed. The dietary fibre content of wheat shorts is lower than in the bran milling fraction, while the fructan content is similar (12). In this study, the effect of wheat shorts and insoluble, water-soluble and ethanol-soluble fractions of shorts on the formation of CA in the hindgut of rats and in portal blood was evaluated. Of particular interest were the formation of CA from soluble AX and the comparison of the effects of wheat fructans with those of inulin-type fructans. Ideally such studies should be performed in humans, but technical difficulties of accessing colon contents hamper the assessment of SCFA production. The rat model used has previously been shown to be useful in the prediction of carbohydrate fermentation and bulking capacity in man (22).

The water-soluble fraction, in which 59% of the dietary fibre was composed of AX and 17% of fructan, caused increased formation of propionic acid, especially compared with the formation of acetic acid, as seen in the proportions of these acids throughout the hindgut and the lower ratio of acetic to propionic acid in portal blood (acetic:propionic acid = 7.6 vs 12–18 for the other test diets). This may be considered favourable as a decrease in this ratio has been proposed as one mechanism behind the hypolipidemic effects of inulin and oligofructose (23). These results agree with our recent study on the fermentation pattern of dietary-fibre-rich by-products from the wet fractionation of wheat to starch and gluten. In that study, two water-soluble dietary fibre fractions, which were rich in AX and fructan, led to a lower ratio of acetic to propionic acid in portal blood than two fractions with a lower solubility containing about the same amount of AX but less fructan (7.6 and 8.6 vs 15 and 14, respectively) (24). Several other studies have suggested that AX and AXOS promote the formation of propionic acid, although the results available in the literature are not conclusive (10, 11, 25, 26). The higher formation of propionic acid from the water-soluble fraction (59% AX and 17% fructan) than from the ethanol-soluble fraction (1% AX and 67% fructan) could both be due to a higher potential of AX than of fructan to form propionic acid and due to an additive effect of AX and fructan.

Inulin-derived oligofructose was used in the reference diet mainly for comparison of the properties of inulin- and graminan-type fructans. As a large amount of raffinose was present in the ethanol-soluble diet, the comparison with oligofructose was facilitated by designing the OR diet so as to have the same ratio of oligofructose to raffinose as of wheat fructans to

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Table 6. Levels (μmol/g wet content) and proportions (%) of acetic, propionic and butyric acids

	Level						Proportion					
	Shorts	Insoluble	Water-soluble	Ethanol-soluble	OF	OR	Shorts	Insoluble	Water-soluble	Ethanol-soluble	OF	OR
Caecum	*n* = 7	*n* = 7	*n* = 7	*n* = 7	*n* = 6	*n* = 5	*n* = 7	*n* = 7	*n* = 7	*n* = 7	*n* = 6	*n* = 5
Acetic	52 ± 3.3a	42 ± 2.2ab	32 ± 5.2ab	44 ± 7.4ab	25 ± 3.8b	28 ± 8.1ab	69 ± 1.1a	69 ± 0.8a	59 ± 1.8b	66 ± 1.8a	66 ± 1.4a	57 ± 2.6b
Propionic	9.1 ± 0.6ab	8.8 ± 0.3ab	17 ± 3.7a	13 ± 1.6a	7.1 ± 1.1b	7.4 ± 1.7b	12 ± 0.6d	15 ± 0.5cd	30 ± 1.7a	21 ± 1.9b	19 ± 1.8bc	16 ± 1.8bcd
Butyric	14 ± 1.3a	9.5 ± 1.0abc	6.1 ± 1.0c	8.6 ± 1.5abc	6.0 ± 1.3bc	13 ± 3.0ab	19 ± 1.1b	16 ± 0.9bc	11 ± 1.2c	13 ± 1.3bc	15 ± 1.4bc	27 ± 2.4a
Total	75 ± 4.7a	60 ± 3.4ab	55 ± 9.3ab	65 ± 9.9ab	39 ± 5.8b	49 ± 12ab						
Proximal colon	*n* = 7	*n* = 6	*n* = 6	*n* = 6	*n* = 4	*n* = 5	*n* = 7	*n* = 6	*n* = 6	*n* = 6	*n* = 4	*n* = 5
Acetic	37 ± 4.5a	29 ± 3.2ab	13 ± 1.5c	17 ± 3.9bc	15 ± 2.0bc	14 ± 1.6bc	69 ± 2.0a	70 ± 1.7a	57 ± 1.2bc	66 ± 1.4ab	67 ± 3.9ab	50 ± 3.9c
Propionic	8.1 ± 1.5	6.3 ± 0.7	6.7 ± 1.0	4.9 ± 0.9	4.0 ± 0.9	5.7 ± 0.7	15 ± 0.4d	15 ± 1.0cd	28 ± 1.2a	20 ± 1.4b	17 ± 1.8bc	20 ± 0.9bcd
Butyric	9.8 ± 2.9a	6.5 ± 1.3abc	3.5 ± 0.5bc	3.5 ± 0.8c	3.8 ± 0.9abc	8.7 ± 1.7ab	16 ± 1.8b	15 ± 1.5b	15 ± 1.7b	14 ± 1.1b	16 ± 2.2b	30 ± 3.2a
Total	55 ± 8.9a	41 ± 4.9ab	23 ± 2.8b	25 ± 5.5b	23 ± 3.4b	28 ± 3.4ab						
Distal colon	*n* = 7	*n* = 7	*n* = 6	*n* = 6	*n* = 3	*n* = 5	*n* = 7	*n* = 7	*n* = 6	*n* = 6	*n* = 3	*n* = 5
Acetic	28 ± 2.2a	19 ± 2.1ab	17 ± 1.8b	15 ± 2.9b	14 ± 2.2b	24 ± 3.6ab	65 ± 1.8a	65 ± 1.0a	62 ± 1.9ab	63 ± 1.4ab	70 ± 2.0a	51 ± 6.2b
Propionic	7.2 ± 0.7a	4.9 ± 0.4ab	5.9 ± 0.5ab	5.0 ± 1.0ab	3.4 ± 0.3ab	9.4 ± 3.2a	16 ± 0.3b	17 ± 0.7b	23 ± 2.1a	21 ± 0.9ab	18 ± 0.8ab	17 ± 1.5ab
Butyric	8.5 ± 1.4ab	5.4 ± 1.0abc	4.0 ± 0.4abc	4.4 ± 1.4bc	2.4 ± 0.2c	20 ± 9.0a	19 ± 1.7b	18 ± 1.5b	15 ± 1.4b	16 ± 1.7b	12 ± 1.3b	32 ± 6.1a
Total	44 ± 3.8a	30 ± 3.4ab	27 ± 2.2ab	24 ± 5.2b	20 ± 2.6b	53 ± 15ab						

Note: Results are given as means ± SEM. Means that do not share a letter are significantly different ($P < 0.05$).
OF, oligofructose; OR, oligofructose and raffinose.

raffinose. However, the formation of CA in rats fed the ethanol-soluble diet was more similar to that of the OF diet than the OR diet. In particular, the proportions of butyric acid were lower and the proportions of acetic acid higher with the ethanol-soluble and OF diets. Furthermore, the ethanol-soluble diet led to significantly higher caecal pools of propionic, isobutyric and isovaleric acids, and more isobutyric acid in portal blood than the OR diet, but not compared with the OF diet. A lower caecal tissue weight was found in rats fed the ethanol-soluble diet than in those fed the OR and OF diets. There may be several possible explanations for this. First, the different structure and DP of the wheat fructans compared with the inulin-type fructans in the OR diet may induce different reactions in the rats. More propionic acid was produced *in vitro* from wheat stem and barley grain fructans (DP fractions 5–15) than from oligofructose (27), which is consistent with the higher caecal pool of propionic acid in the ethanol-soluble group than the OR group. While the production of butyric acid *in vitro* was similar to that of oligofructose and inulin (DP 10–60) for DP fractions 5–15, the low DP fractions (DP 3–6) produced less butyric acid. 27% of the fructans in the ethanol-soluble fraction were of DP 3–4, which may explain the lower caecal and colonic proportions of butyric acid in the ethanol-soluble group than the OR group. Secondly, the ethanol-soluble fibre fraction contained 17.5% units of other dietary fibre, 8.5 of which were composed of glucose. As a consequence, the amount of fructan and raffinose was lower in this diet than in the OR diet. These additional components may also have modified the effect of combining raffinose with fructan. Thirdly, it cannot be excluded that some protein from the ethanol-soluble fraction reached the colon. Proteins are known to result in branched SCFA during fermentation (28).

The increased proportion of butyric acid with the addition of raffinose to the OF diet, as indicated by ratios of acetic, propionic and butyric acid in the caecum of 57:16:27 and 66:19:15 with the OR and OF diets, respectively, was not in line with earlier studies with a similar design, which showed caecal formation of acetic, propionic and butyric acids at ratios of 69:15:15 for raffinose and 47:22:31 for oligofructose (5, 19). It may thus be speculated that the combination of oligofructose and raffinose resulted in a synergistic effect that increased the proportion of butyric acid. Such a synergistic effect of combining different kinds of dietary fibre has been seen, for example, with a diet containing pectin (an acetic acid producer) and guar gum (a propionic acid producer), which together increased the formation of butyric acid (6). The reason for the different results for the pure OF diet is not known, but one reason for the different results seen in the two studies may be different adaptation times (29).

Table 7. Levels (μmol/g) of short-chain fatty acids in portal blood

	Shorts	Insoluble	Water-soluble	Ethanol-soluble	OF	OR
Acetic	1193±56a	872±37b	929±60ab	1125±83ab	940±57ab	913±91ab
Propionic	97±13ab	72±7.5ab	163±47a	103±13ab	76±8.5ab	65±14b
Isobutyric	13±1.5a	9.3±0.8ab	6.5±0.7bc	10±0.6ab	6.8±1.1bc	5.2±1.4c
Butyric	91±17	52±8.4	47±13	49±9.0	58±21	63±17
Isovaleric	15±2.0a	11±1.1ab	7.0±0.7b	11±0.8ab	9.3±1.3ab	6.4±1.5b
Total	1409±75a	1016±49b	1152±112ab	1298±97ab	1090±58ab	1053±118ab
Acetic: Propionic	13±1.4a	13±1.0a	7.6±1.2b	12±1.0ab	13±1.5a	18±5.6a

Note: Results are given as means±SEM, $n=5$–7. Means that do not share a letter are significantly different ($P<0.05$).
OF, oligofructose; OR, oligofructose and raffinose.

The rats in the OR group had the highest caecal tissue weight, but this was not positively correlated to the caecal content, as in the other groups. A correlation between caecal tissue weight and the caecal content has also been reported in an earlier study and explained by mechanical stress (5). A possible explanation for this discrepancy is that the gas detected in the caecum of several rats from the OR group contributed to the growth of the caecal tissue.

The fractionation process was performed with the addition of xylanase in order to achieve an adequate yield of AX in the water-soluble fraction. AX and AXOS have been shown to have different carboxylic-acid-forming and prebiotic properties depending on the DP (9, 10), suggesting that the water-soluble AXs in the water-soluble fraction are likely to be different from those in the untreated shorts. The architecture (three-dimensional structure) is another factor that has been proposed to differ in whole material and in purified fractions (30, 31). Another effect of the fractionation process was that the glucose-containing polymers (cellulose and beta-glucan) became more available to fermentation, as shown by the lower excretion of glucose-containing polysaccharides in rats fed both the insoluble and the water-soluble diets than in those fed the shorts diet.

In conclusion, the different fractions of wheat shorts affected the formation of CA in the hindgut and portal blood of rats in different ways. Of particular interest was the finding that the water-soluble fraction, with a high content of AX and fructan, led to increased formation of propionic acid in the hindgut and a lower ratio of acetic to propionic acid in the portal blood of rats. The results further indicate that the wheat fructans produced more propionic acid and less butyric acid than oligofructose. Another interesting observation was the increased formation of butyric acid in rats fed the OR diet, containing oligofructose and raffinose, compared with those fed the diet containing only oligofructose, possibly suggesting a synergistic effect of these carbohydrates.

Conflict of interest and funding

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