

Dietary fibre-rich oat-based products affect serum lipids, microbiota, formation of short-chain fatty acids and steroids in rats

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Wistar rats (ten per group) were fed either an oat-free control diet or a dietary fibre-rich test diet containing 500 g oat-based products/kg for 6 weeks. The oat-based products, containing 4–128 g/kg resistant starch, 30–92 g/kg β -glucan and 122–304 g/kg total dietary fibre, were oat flour extrudate, flour/Novelose (commercial resistant starch) extrudate (80:20 w/w), oat bran, bran/Novelose extrudate (80:20 w/w) and autoclaved oat flour. Serum total cholesterol decreased in the groups fed flour, flour/Novelose and bran/Novelose ($P < 0.05$). In most of the test groups, count numbers of bifidobacteria were higher ($P < 0.001$) and of coliforms were lower ($P < 0.05$). The mass of the caecum walls and contents was greater in groups fed Novelose- and bran-containing diets ($P < 0.005$). In all the test groups, pH values were lower in the intestinal contents ($P < 0.001$), and caecal concentrations of acetate ($P < 0.001$), propionate ($P < 0.05$), butyrate ($P < 0.005$) and total SCFA ($P < 0.001$) were higher. The lowest concentrations of steroids were found in rats fed the autoclaved flour. In the other test groups, more bile acids appeared in the caecal ($P < 0.001$) and colonic contents ($P < 0.005$), as well as in the faeces, at week 6 ($P < 0.001$). The highest bile acid excretion was found after feeding bran-containing diets. In the intestinal contents of all the test groups, more primary bile acids ($P < 0.001$) appeared than in the control group. The excretion of steroids increased within the experimental period. Using extrusion technology, dietary fibre-rich oat-based products, which have beneficial physiological effects in rats, can be produced. Oat flour and bran are excellent sources for the preparation of directly edible oat products. Their nutritional properties can be further improved by the addition of resistant starch.

Oat: Physiological effect: Short-chain fatty acid: Steroid: Rat

Oat (*Avena sativa* L.) products are well accepted in human nutrition. They contain relatively high concentrations of protein, lipids, vitamins, antioxidants, phenolic compounds and minerals (Hampshire, 1998; Emmons & Peterson, 1999; Zadernowski *et al.* 1999; Peterson, 2001; Panfili *et al.* 2003). Furthermore, oat is an excellent source of different dietary fibre (DF) types, such as mixed-linked (1 \rightarrow 3), (1 \rightarrow 4)- β -D-glucan (here referred to as β -glucan), arabinoxylans and cellulose. DF are essential components of human nutrition. A high intake of DF is positively related to several preventive medical and nutritional effects (Spiller, 2001). However, the daily intake of DF is below the recommended concentration of at least 30 g for adults in most of the industrialised countries (Cummings & Frølich, 1993; Deutsche Gesellschaft für Ernährung, 2004).

Among the DF components of oat, β -glucan plays an important role because of its viscosity and functional properties in the gastrointestinal tract (Doubliner & Wood, 1995; Tejinder *et al.* 2000; Mälkki & Virtanen, 2001; Dongowski *et al.* 2005). Several effects of β -glucan in the upper intestinal tract are under discussion. It may thus lower the postprandial blood glucose and insulin responses in normal individuals (Braaten *et al.* 1991). Furthermore, β -glucan has cholesterol-lowering effects in hypercholesterolaemic humans and animals (Kahlon *et al.* 1993;

Braaten *et al.* 1994; Hecker *et al.* 1998; Kalra & Jood, 2001; Kerckhoffs *et al.* 2002; Delaney *et al.* 2003b). Interactions between β -glucan and bile acids (BA) in the small intestine are the precondition for the greater excretion of BA. A higher excretion of BA causes their increased synthesis from cholesterol, as shown during the consumption of β -glucan from oat bran (Andersson *et al.* 2002). This effect is connected with a lowering of blood cholesterol level in the presence of β -glucan-rich diets (Zhang *et al.* 1992).

β -Glucan is practically completely fermented in the caecum and colon by the microflora. Major fermentation products are SCFA, which are important for a healthy colonic mucosa (Daniel *et al.* 1997; Topping & Clifton, 2001).

In oat products such as bran, the native three-dimensional DF-rich cell wall architecture may be partly preserved during passage through the intestinal tract. It is well known that cereal bran significantly accelerates transit time and increases daily faecal weight in humans (Lupton *et al.* 1993).

Another DF component present in certain cereal (starchy) products is so-called resistant starch (RS). RS is defined as the starch, or starch degradation products, that is not absorbed in the small intestine of healthy individuals. It is a major substrate for fermentation by intestinal bacteria and is a good source of butyrate (Asp

et al. 1996; Brouns *et al.* 2002). There are two principal ways to increase the proportion of RS in cereal products: the addition of commercially available RS preparations or special treatments (e.g. the controlled heating/cooling) of starch-containing materials. It was thus possible to generate RS in barley products (Huth *et al.* 2000) but not in oat flour (Gebhardt *et al.* 2004) by extrusion.

Food-processing such as extrusion can influence the digestibility of starch in cereal products (Bornet, 1993). There is scant information on the influence of the composition of extruded products on their physiological effects. Chang *et al.* (2002) reported on the hypocholesterolaemic properties of extrudates prepared from mixtures of cassava starch and Novelose or oat fibre. However, the proportions of the components in the extrudates were not given.

The objective of the present study was to evaluate the physiological and nutritional effects of DF-rich oat-based products (e.g. extrudates) in rats. The oat-based products tested differed in their contents of individual DF such as β -glucan and RS, as well as of soluble and insoluble DF. A higher consumption of such oat products should improve the intake of DF and other essential nutrients. Oats are a cereal with an important nutritional value but are used as food at a relatively low level in most countries. Our hypothesis was that the composition and pre-treatment of oat-based products would be important for the direct or indirect physiological effects of oat products in the intestinal tract.

Materials and methods

Source materials and oat products

Oat flour was prepared from commercial oat kernels by milling. Oat bran was obtained from Peter Kölln Köllnflockenwerke (Elmshorn, Germany). Novelose 330 is a commercial RS product from National Starch & Chemical (Hamburg, Germany).

The oat-based extrudates oat flour (product F), oat flour/Novelose (product FN; 80:20 w/w) and oat bran/Novelose (product BN; 80:20 w/w) were prepared in the twin-screw extruder ERMAFA DS 6-0 (ERMAFA Kunststofftechnik, Chemnitz, Germany) using the following conditions: moisture content 25%, mass temperature 150°C, screw speed 200 rpm and dosage 100 kg/h. Oat

product B consisted of untreated oat bran. Furthermore, oat flour was treated twice for 60 min at 140°C and at a moisture content of 25% in the Sanoclav autoclave (Adolf Wolf, Bad Überkingen-Hausen, Germany) (product F#).

Experimental diets

The experimental diets were prepared as pellets in the German Institute of Human Nutrition, Potsdam-Rehbruecke (Table 1). The oat-based test diets contained 500 g/kg oat products and only 130 g/kg wheat starch (partial replacement of the wheat starch used in the control diet).

Rat experiment

Young male Wistar rats (Tierzucht Schönwalde GmbH, Schönwalde, Germany) were housed individually in temperature- and humidity-controlled cages (22°C and 55%) on a normal 12 h light/dark cycle. During the adaptation period, all rats were fed the control diet for 7 d after arrival (week 0). Then they were randomly divided into six groups of ten rats each and fed the control diet or the oat-based test diets for 6 weeks. The rats had free access to food and water during the experiment.

Growth of the rats and food and water intake were determined weekly. To determine the steroid levels, faeces were collected completely for 24 h at weeks 0, 2, 4 and 6. Blood samples were taken from eye plexus of ether-anaesthetised rats, after a 16 h fast, for analysis of serum lipids at weeks 0 and 6. After 6 weeks of the diet, rats were killed, and selected organs (e.g. ileum, caecum, colon) and the contents of ileum, caecum and colon were prepared for analysis. The colon was divided into the proximal and the distal part. SCFA, steroids, DM, pH values and RS were analysed in the intestinal contents. The microbial counts were determined at the end of the experiment in fresh faecal samples collected directly from the anus.

The rat experiment was carried out in the Max-Rubner-Laboratory of the German Institute of Human Nutrition, Potsdam-Rehbruecke in 2003. The experimental protocol was performed according to international and national guidelines. All treatments and diets were formally approved by the Animal Welfare Committee of the State Brandenburg (Ministry for Agriculture,

Table 1. Composition of the control diet and the oat-based diets (g/kg)

Diet	Control diet	Diet F	Diet FN	Diet B	Diet BN	Diet F#
Casein*	200	200	200	200	200	200
Wheat starch†	630	130	130	130	130	130
Oat-based product	0	500	500	500	500	500
Sunflower-seed oil‡	50	50	50	50	50	50
Microcrystalline cellulose§	50	50	50	50	50	50
Mineral mixture	50	50	50	50	50	50
Vitamin mixture¶	20	20	20	20	20	20

F, oat flour; FN, oat flour/Novelose; B, oat bran; BN, oat bran/Novelose; F#, oat flour (autoclaved).

* Casein, edible (Bayerische Milchindustrie e.G., Landshut, Germany).

† Hermann Kröner GmbH Co. KG, Ibbenbüren, Germany.

‡ Bröilo Europe GmbH/Ölmühle GmbH + Co., Hamm, Germany.

§ Vivapur (Heweten 20) (Rettenmaier & Söhne GmbH + Co., Ellwangen-Holzalmühle, Germany).

|| Altromin GmbH, Lage, Germany. The composition of the mineral mixture was (mg/kg diet): Ca, 7300; P, 4900; Na, 2000; Mg, 450; K, 6000; S, 530; Cl, 3200; Fe, 150; Mn, 90; Zn, 20; Cu, 4; I, 0.4; Mo, 0.2; F, 4; Se, 0.2; Co, 0.1.

¶ Altromin GmbH, Lage, Germany. The composition of the vitamin mixture was (per kg diet): vitamin A, 15 000 IE; vitamin D₃, 500 IU; vitamin E, 150 mg; vitamin K₃, 10 mg; vitamin B₁, 20 mg; vitamin B₂, 20 mg; vitamin B₆, 15 mg; vitamin B₁₂, 0.03 mg; niacin, 50 mg; pantothenic acid, 50 mg; folic acid, 10 mg; biotin, 0.2 mg; choline chloride, 1000 mg; *p*-aminobenzoic acid, 100 mg; inositol, 100 mg; vitamin C, 20 mg; methionine, 3500 mg.

Environmental Protection and Regional Planning), Germany (permissions 32/48-3560-0/3).

Analytical methods

Insoluble and soluble DF fractions were analysed by the enzymatic-gravimetric Association of Official Analytical Chemists method (Prosky *et al.* 1988). Non-digestible oligosaccharides were estimated in the supernatant after coagulation of the soluble DF fraction with ethanol using HPLC with refractive index detection. RS was measured by a modified Englyst-method (Englyst *et al.* 1992) after hydrolysis of the digestible starch and extraction of the hydrolysis products with 80% ethanol v/v. The RS was dissolved in 1 M-NaOH, hydrolysed with amyloglucosidase and determined enzymatically as released glucose. The total DF content was calculated as the sum of soluble and insoluble DF, non-digestible oligosaccharides and RS.

Total fat was determined by the Weibull–Stoldt method. Protein was estimated by a modified Kjeldahl method ($N \times 6.25$). The total starch content was determined enzymatically using amyloglucosidase and the Boehringer glucose kit after extraction in 1 M-NaOH. The glucose released was analysed with the hexokinase/glucose-6-phosphate dehydrogenase kit from Boehringer (Mannheim, Germany).

For the determination of β -glucan, sample material was suspended in phosphate buffer (pH 6.5) and mixed for 5 min at 100°C. The diluted suspension was hydrolysed with lichenase (Megazyme International, Bray, Ireland) for 60 min at 40°C (McCleary & Mugford, 1997). After dilution and centrifugation (10 min at 1500g), a part of the supernatant was incubated with β -glucosidase (Megazyme) in acetate buffer at pH 4.5 and 40°C for 15 min. The glucose liberated was determined enzymatically.

Water uptake of the oat-based products was determined using the capillary suction method (Arrigoni *et al.* 1987) at 20°C for 15 min and was expressed as g H₂O/g substance.

Before determining the acid extract viscosity in the Ubbelohde viscometer (at 25.0°C), 50 g of the oat-based products were extracted with 500 ml 0.2 M-KCl/HCl at pH 1.5 and 40°C for 2 h (Huth *et al.* 2000). The acid extract viscosity is given as relative viscosity ($\eta_{rel} = \eta/\eta_0$, where η is the viscosity of the extract and η_0 is that of the solvent).

To evaluate the molecular weight, β -glucan was extracted from the oat-based products using a modified method of Westerlund *et al.* (1993) by treatment with Termamyl 120L (Novo Nordisk A/S, Copenhagen, Denmark), pancreatin (Merck, Darmstadt, Germany) and a purification procedure (Dongowski *et al.* 2005). The intrinsic viscosity $[\eta]$ was estimated at 25.0°C in an Ubbelohde viscometer (Schott Instruments GmbH, Mainz, Germany). The β -glucan preparations were dissolved in water and then centrifuged for 10 min at 2000g. Relative viscosity was determined on the basis of flow times at different concentrations. The intrinsic viscosity $[\eta]$ was determined by extrapolating the calculated specific viscosity ($\eta_{sp} = \eta_{rel} - 1$) against the concentration of β -glucan using the Huggins equation. The average molecular weight (M_w) was calculated using the Mark–Houwink equation $[\eta] = k \times M_w^a$, where the factor k and the exponent a are dependent on the nature of the macromolecules and the solvent. In line with Vårum *et al.* (1991), $k = 0.00067$ and $a = 0.75$ were used for calculations.

Triacylglycerols, total cholesterol and HDL- and LDL-cholesterol were measured enzymatically in serum using commercial

kits (Olympus Diagnostica GmbH, Hamburg, Germany). SCFA in the caecal contents were analysed by gas chromatography using a modified method of Brighenti (1997) as previously described (Dongowski *et al.* 2002).

The microbial counts were determined as described by Sembries *et al.* (2003). Approximately 0.2 g fresh collected faeces was immediately placed into pre-weighed tubes and diluted with pre-reduced buffered peptone water. In duplicate, 0.05 ml of each dilution was plated on non-selective and selective media. Columbia blood agar (BioMérieux, Nürtingen, Germany), Endo agar (BioMérieux) and Rogosa agar (Fluka, Taufkirchen, Germany) were incubated aerobically at 37°C to determine the total aerobes (for 48 h), coliform bacteria (for 24 h) and aerobic lactobacilli (for 48 h), respectively. Numbers of total anaerobes, *Bacteroides* and *Bifidobacterium* sp. were determined after 48 h anaerobic incubation of Columbia blood agar, Columbia blood agar supplemented with neomycin (0.1 g/l; Fluka) and sodium deoxycholate (0.2 g/l; Fluka), and Haenel/Müller-Beuthow medium. The composition of this medium was (g/l): peptone (10), yeast extract (7), NaCl (3), Na₂HPO₄ (2), agar (10), cystin (0.5), cystein (0.5), sodiumazide (0.1), bromocresol green (0.0125), neutral red (0.0025), 2.5 ml saline B (40 g/l MgSO₄·7 H₂O, 2 g/l FeSO₄·7 H₂O, 2 g/l NaCl, 2.35 g/l MnSO₄·2 H₂O), respectively. Faecal microbial counts were expressed as log₁₀ colony-forming units/g.

The procedures for extraction and purification from freeze-dried intestinal contents and faecal materials, as well as the analysis of BA by HPLC using pre-column derivatisation with 4-bromomethyl-7-methoxycoumarin and fluorescence detection, and of neutral sterols (NS), using high performance TLC are given elsewhere (Dongowski *et al.* 2003).

Statistics

Data are presented as mean values with their standard errors. Data were analysed by one-way ANOVA with dietary groups as fixed factors. Differences between the control group, given an oat product-free diet, and the test groups, given the DF-rich oat-based diets, were evaluated by Dunnett's *t* test and Dunnett's T3 test for multiple *post hoc* comparisons. When variances were heterogeneous, data were log-transformed before analysis (concentrations of cholesterol in caecal contents; taurocholic and taurochenodeoxycholic acid in colonic contents; α -muricholic acid at weeks 2 and 4, chenodeoxycholic acid at weeks 4 and 6, coprostanol and cholestanone at week 4 in faeces; concentrations of propionic and *n*-valeric acid in caecal contents at week 6; weights of ileal contents at week 6; DM content of faeces at week 6; count numbers of *Bacteroides* at week 6; food and water intake at weeks 1 and 2). Differences with a value $P < 0.05$ were considered significant. Statistical analysis was performed using Statistical Package for Social Sciences software SPSS 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Oat-based products and experimental diets

A series of oat-based products consisting of oat flour, oat bran and/or Novelose 330 in extruded, autoclaved or untreated form were used for the experiments. The composition of these products is given in Table 2. The oat-based products differed in their contents of β -glucan (30–92 g/kg), RS (4–128 g/kg), soluble

Table 2. Composition and properties of the oat-based products

Oat-based product	F	FN	B	BN	F#
Ingredient (g/kg)					
Oat flour	1000	800			1000
Oat bran			1000	800	
Novelose 330		200		200	
Analysed composition (g/kg)*					
Starch	586	567	369	435	529
Resistant starch	4	128	5	114	58
Protein	159	129	192	164	145
Lipids	61	50	89	59	62
Soluble DF	39	26	90	72	47
Insoluble DF	47	55	129	100	33
Non-digestible oligosaccharides	32	27	13	18	18
Total DF†	122	236	237	304	156
β-Glucan	41	30	92	69	39
Functional properties					
Water uptake (g H ₂ O/g)	5.69	4.59	2.88	4.63	2.57
Acid extract viscosity‡	67.5	51.0	165.4	115.1	4.5
Molecular weight of β-glucan (kDa)§	94.8	145.8	132.4	131.2	49.1

F, oat flour; FN, oat flour/Novelose; B, oat bran; BN, oat bran/Novelose; F#, oat flour (autoclaved).

* Mean values of at least duplicates; for β-glucan and resistant starch, *n* 4–6.

† Sum of soluble and insoluble dietary fibre, non-digestible oligosaccharides and resistant starch.

‡ Relative viscosity.

§ Calculated from intrinsic viscosity using the Mark–Houwink equation.

DF (26–90 g/kg), insoluble DF (33–129 g/kg) and non-digestible oligosaccharides (13–32 g/kg), as well as of lipids, protein and starch. In detail, β-glucan-rich were the bran-containing products and RS-rich were the Novelose-containing products. However, in autoclaved flour, a higher RS content was also present. The highest soluble, insoluble and total DF values were measured in the bran-containing samples. The proportion of the non-digestible oligosaccharide fraction was relatively high in the oat-based products. The source of most of these non-digestible oligosaccharides is the oligofructose (inulin) fraction of oat. Furthermore, some oligosaccharides, soluble in approximately 80% ethanol v/v, may be formed from polymeric dietary fibre components during the Association of Official Analytical Chemists' method.

The oat-based products also differed in several of their functional properties. Thus, water uptake was highest in extruded and lowest in autoclaved flour. Extrusion of the bran–Novelose mixture resulted in an increased water uptake compared with the untreated bran (Table 2).

The acid-extract viscosity was highest in the extracts from bran-containing oat products and extremely low in the extract from the autoclaved product (Table 2). After the enzymatic removal of starch and protein from the oat-based products, β-glucan preparations were isolated. The molecular weights of these preparations, calculated from the intrinsic viscosities using the Mark–Houwink equation, were between approximately 95 and 146 kDa (Table 2). However, the β-glucan preparation isolated from autoclaved oat flour had a molecular weight of only 49 kDa. Functional properties of the oat products such as water-binding or viscosity may play an important role during their passage through the gastrointestinal tract.

Compared with the control diet, the oat-based diets contained more soluble, insoluble and total DF as well as more β-glucan, but less wheat starch. In Novelose-containing diets and in the diet containing autoclaved oat flour, more than 30 g/kg RS was found. Furthermore, 50 g/kg microcrystalline cellulose was present in all the experimental diets (Table 1).

Behaviour, food and water intake and weight gain of the rats

The diets were well accepted by the animals. There were no treatment-related changes in behaviour or appearance of the rats during the experiment, and all the rats remained healthy during the experimental period.

No differences in food intake were found between the groups. The food intake increased in all groups up to week 5. The following food intakes were measured (per rat and week): week 1, 96.8 (SEM 1.2) g; week 2, 121.4 (SEM 1.6) g; week 3, 114.4 (SEM 1.3) g; week 4, 133.4 (SEM 2.1) g; week 5, 135.4 (SEM 2.1) g; week 6, 119.1 (SEM 1.7) g. The total food intake during the experiment was 720.6 (SEM 8.6) g per rat. The water intake increased at least up to week 4 in all groups. In groups consuming the oat-based diets, a higher water intake was measured compared with the control group (Fig. 1). The highest water intake was found in rats fed the bran-containing diet B. There were no differences in weight gain among the groups. The weight of the rats increased as follows: week 0, 86.3 (SEM 0.6) g; week 2, 166.3 (SEM 1.4) g; week 4, 243.0 (SEM 2.2) g; week 6, 303.8 (SEM 3.3) g. Food efficiency that means weight gain (in g) per consumed diet (in g) was similar between the groups.

Serum lipids

At week 0, the following characteristics were found: 2.11 (SEM 0.13) mmol/l total cholesterol; 1.59 (SEM 0.10) mmol/l HDL-cholesterol; 0.46 (SEM 0.07) mmol/l LDL-cholesterol; 0.49 (SEM 0.08) mmol/l triacylglycerols. Because rats had normal serum lipid concentrations, changes during the experiment were relatively low. The differences in selected serum lipids between weeks 6 and 0 are shown in Table 3. Both total and LDL-cholesterol decreased more strongly in most of the groups fed the oat-based diets than in the control group. Triacylglycerols were not uniformly affected by the diet.

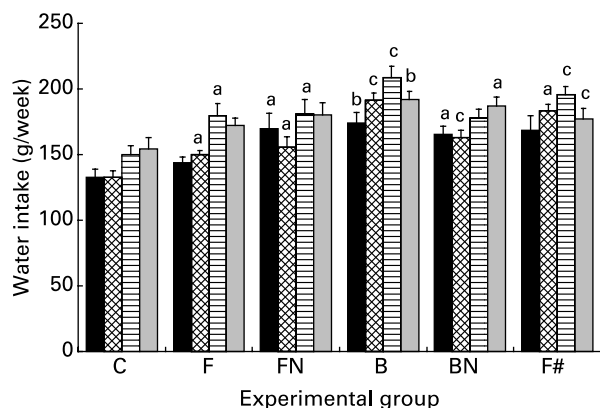


Fig. 1. Water intake of rats (g/week) fed the control diet C or the oat-based diets F, FN, B, BN and F# during the experiment. Values are means of ten rats with their standard errors shown by vertical bars. Mean values were significantly different from those of the control group: ^a $P < 0.05$, ^b $P < 0.005$, ^c $P < 0.001$. ■, week 1; ▨, week 2; ▩, week 4; □, week 6. For details of diets, see p. 1013 and Table 1.

Microbial counts

The numbers of aerobic and anaerobic micro-organisms were higher in rats fed the oat-based diets at week 6. Whereas counts of coliforms were higher in the controls, more bifidobacteria were found in groups fed the oat-based diets. These differences were greater than one order of magnitude in some groups. The numbers of *Bacteroides* and *Lactobacillus* did not differ between the groups (Fig. 2).

Organ mass, intestinal contents and faecal mass

When the rats were killed at week 6, selected organs and intestinal contents were removed. Macroscopic alterations were not observed in the organs of any of the rats. There was no difference in the weights of liver, spleen, stomach, and jejunal and ileal walls between the groups at the end of the experiment. The masses of the caecal walls were, however, significantly greater in most of the groups fed the oat-based diets (Table 4).

The wet contents of the caecum and colon were significantly greater, or showed a trend towards being greater, in some test groups. The reason for this effect was the higher content of DF in these diets. Compared with controls, the ileal and caecal contents were up to 50% and 90% higher, respectively, in groups fed the oat-based diets. Likewise, higher amounts of content

were found in the proximal and distal parts of the colon (up to 80% and 50%, respectively) in these groups (Table 4).

In addition, a higher proportion of DM was found in intestinal contents of rats fed the oat-based diets for 6 weeks. Thus, in the caecal contents, DM was 20.45% (SEM 0.90%) in the control group and up to 25.46% (SEM 0.51%) in groups fed the oat-based diet. In the colonic contents (proximal), DM increased from 25.05% (SEM 2.10%) in the control group up to 32.00% (SEM 2.52%) in groups fed the oat-based diets.

RS was not found in the caecal contents of the control group as well as of groups F and B. Small amounts of RS, however, appeared in the caecal contents of the groups fed diets containing Novelose or autoclaved oat flour. RS was absent from the contents of the distal colon in all groups.

At week 6, pH values were significantly lower ($P < 0.001$) in all the caecal and colonic contents of rats fed the oat-based diets compared with controls (Fig. 3). The reason for this effect is the higher formation of SCFA as a result of DF fermentation by the intestinal microflora. In the caecum, the lowest pH values were measured in the bran-fed group. It is interesting that lower average pH values were present in colonic contents if the diet contained Novelose (FN, BN) compared with the corresponding Novelose-free diets (F, B). This points to a stronger fermentation of RS from Novelose in the colon.

The excreted amounts of DM, determined after freeze-drying of whole faeces collected over 24 h, were significantly different between the control group and the groups fed the oat-based diets at weeks 4 and 6. Thus, excretion of DM was as follows at the end of the experiment: control group, 3.09 (SEM 0.08) g/d; group F, 4.54 (SEM 0.25) g/d; group FN, 4.67 (SEM 0.37) g/d; group B, 4.83 (SEM 0.31) g/d; group BN, 4.44 (SEM 0.15) g/d; group F#, 4.19 (SEM 0.24) g/d ($P < 0.001$; in group F#, $P < 0.005$).

SCFA in caecal contents

SCFA were measured in the caecal contents at week 6. The concentrations of acetate, propionate and butyrate were significantly higher in the whole-caecal contents of all groups fed the oat-based diets compared with controls (wet weight basis). In particular, the bran-containing diets were highly fermentable in the caecum. The extruded flour was fermented more slowly in the caecum than was the autoclaved flour (Fig. 4). In all groups, acetate was the dominant SCFA. More acetate was formed in all test groups than in the control group ($P < 0.001$). Significantly higher concentrations of propionate and butyrate were, however, also found in the test groups. Besides higher total amounts of butyrate, the proportion

Table 3. Differences in serum lipids (mmol/l) between week 6 and week 0 of rats fed control or oat-based diets

(Values are means of ten rats with their standard errors)

Diet group	Total cholesterol		LDL-cholesterol		Triacylglycerols	
	Mean	SEM	Mean	SEM	Mean	SEM
Control	0.031	0.048	-0.071	0.023	0.189	0.088
Flour	-0.236 ^a	0.057	-0.112	0.027	-0.099	0.117
Flour/Novelose	-0.219 ^a	0.073	-0.135	0.034	0.081	0.081
Bran	-0.087	0.077	-0.165 ^a	0.025	0.321	0.054
Bran/Novelose	-0.335 ^b	0.058	-0.136	0.029	-0.165 ^a	0.115
Flour (autoclaved)	-0.062	0.065	-0.164 ^a	0.015	0.292	0.072

Mean values were significantly different from those of the control group: ^a $P < 0.05$, ^b $P < 0.005$.

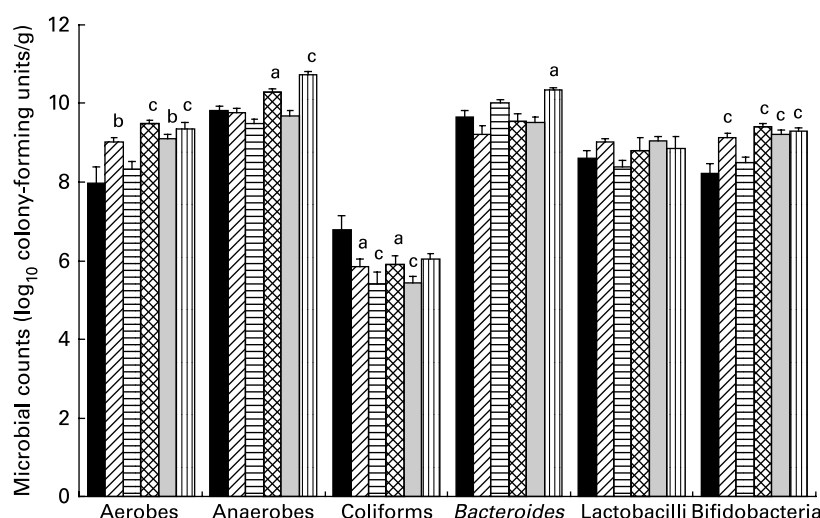


Fig. 2. Microbial counts (\log_{10} colony-forming units/g) in the fresh faecal samples of rats fed the control diet C or the oat-based diets F, FN, B, BN and F# for 6 weeks. Values are means of six rats with their standard errors shown by vertical bars. Mean values were significantly different from those of the control group: ^a $P < 0.05$, ^b $P < 0.005$, ^c $P < 0.001$. ■, C; ▨, F; ▩, FN; ▤, B; ▥, BN; ▦, F#. For details of diets, see p. 1013 and Table 1.

of butyrate increased in all groups fed the oat-based diets. The highest proportion of butyrate was found in the caecal contents of groups F (25.5%), BN (23.7%) and B (19.7%). The valerates were present in only relatively low amounts. The total SCFA concentration of the caecal contents was: control group, 156.1 (SEM 9.9) μmol ; group F, 313.9 (SEM 15.0) μmol ; group FN, 420.5 (SEM 24.9) μmol ; group B, 590.4 (SEM 37.3) μmol ; group BN, 609.3 (SEM 54.3) μmol ; group F#, 345.5 (SEM 33.1) μmol ($P < 0.001$ in all test groups).

Oat bran and Novelose were the best substrates for the microbial production of SCFA. It seems that the autoclaved flour was fermented more quickly than the extruded flour. More SCFA were formed in the caecum of rats given the bran-containing diets. In these dietary groups, more butyrate was also found. The addition of Novelose 330 improved SCFA formation only in combination with oat flour.

Steroids in intestinal contents and faeces

Concentrations of BA and NS were determined in the caecal and colonic contents at week 6 and in faeces at weeks 0, 2, 4 and 6. On a DM basis, the amount of total BA increased from caecum contents to faeces in all groups.

Besides the individual BA cholic acid, deoxycholic acid, chenodeoxycholic acid, lithocholic acid, and their metabolites such as 7-ketodeoxycholic acid, 12-ketolithocholic acid, hyodeoxycholic acid, ursodeoxycholic acid and α - and β -muricholic acid, several tauroconjugates (taurocholic acid, taurodeoxycholic acid, taurochenodeoxycholic acid) were present in the intestinal contents. Whereas 22.53% tauroconjugated BA were found in the caecal contents of the control group, and 16.26–17.98% in the oat-based groups ($P < 0.001$), the proportion of tauroconjugates in the colonic contents was only 4.12% in the control group and 1.08–3.25% in the test groups ($P < 0.05$). In the faeces, exclusively free BA were present.

In whole-caecal contents, the total BA concentration was: control group, 10.51 (SEM 0.68) μmol ; group F, 16.31 (SEM 0.45) μmol ; group FN, 22.29 (SEM 1.14) μmol ; group B, 18.45 (SEM 0.79) μmol ; group BN, 21.38 (SEM 0.62) μmol ; group F#, 13.38 (SEM 0.33) μmol . With exception of the diet containing the autoclaved flour ($P = 0.067$), significantly more total BA were present in groups given the oat-based diets compared with controls ($P < 0.001$). In whole-colonic contents, the total BA concentration in the control group was 2.60 (SEM 0.28) μmol , whereas that in the groups fed the oat-based diets ranged between 4.23 (SEM 0.34) μmol and 6.17 (SEM 0.34) μmol ($P < 0.05$).

Table 4. Weights of intestinal walls and contents (g wet weight) of rats fed control or oat-based diets for 6 weeks (Values are means of ten rats with their standard errors)

Diet group	Caecum				Colon (proximal)				Colon (distal)			
	Walls		Contents		Walls		Contents		Walls		Contents	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	0.808	0.068	5.804	0.278	0.697	0.058	1.023	0.119	0.477	0.052	1.452	0.124
Flour	0.918	0.050	6.189	0.240	0.832	0.033	1.532	0.095	0.474	0.040	1.618	0.142
Flour/Novelose	1.177 ^c	0.076	11.101 ^c	0.569	0.797	0.055	1.416	0.127	0.528	0.025	2.194 ^c	0.121
Bran	1.177 ^c	0.055	7.745 ^b	0.512	0.895 ^c	0.024	1.564	0.161	0.565	0.030	1.950 ^a	0.136
Bran/Novelose	1.230 ^c	0.065	8.978 ^c	0.530	0.850	0.040	1.135	0.101	0.557	0.031	1.608	0.079
Flour (autoclaved)	1.089 ^a	0.041	5.963	0.229	0.667	0.036	1.828 ^b	0.219	0.494	0.023	1.822	0.130

Mean values were significantly different from those of the control group: ^a $P < 0.05$, ^b $P < 0.005$, ^c $P < 0.001$.

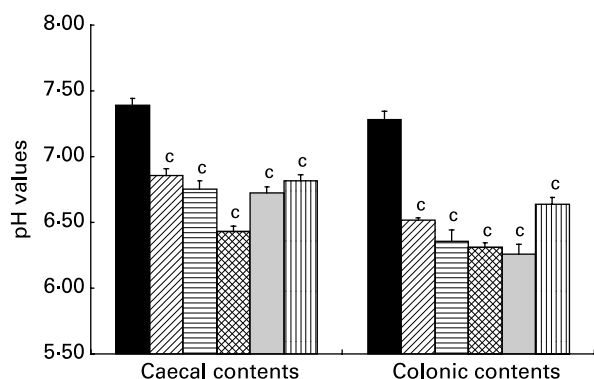


Fig. 3. pH values of the caecal and colonic contents of rats fed the control diet C or the oat-based diets F, FN, B, BN and F# for 6 weeks. Values are means of ten rats with their standard errors shown by vertical bars. Mean values were significantly different from those of the control group: ^c*P*<0.001. ■, C; ▨, F; ▩, FN; ▧, B; □, BN; ▤, F#. For details of diets, see p. 1013 and Table 1.

During the experiment, the excretion of BA increased to different degrees in each of the groups. Compared with controls, significantly more BA were excreted per day in the groups fed the oat-based diets at weeks 4 and 6 (Fig. 5). The excretion was highest in the bran group and lowest in the group fed the autoclaved oat flour. The partial replacement of oat products by Novelose did not improve the excretion of BA. Concentrations of the individual BA in the faeces of rats fed the control and oat-based diets for 6 weeks are given in Table 5. In all the test groups, significantly more cholic acid, 7-ketodeoxycholic acid, chenodeoxycholic acid, α -muricholic acid, β -muricholic acid and ursodeoxycholic acid were measured. These BA belong to the so-called primary BA, having a hydroxyl group at C atom 7 of the steroid nucleus. Primary BA are formed in the liver and dominate in the bile and small intestine. During passage through the lower intestinal tract, a proportion of the primary BA is converted to secondary BA by the action of bacterial enzymes. Secondary BA have no hydroxyl or keto group at C atom 7 of the steroid nucleus.

In whole-caecal and proximal colonic contents, more primary as well as more secondary BA were present in rats fed the oat-based diets (Fig. 6). High concentrations of primary BA were found in the whole-caecal contents of groups B and BN, in

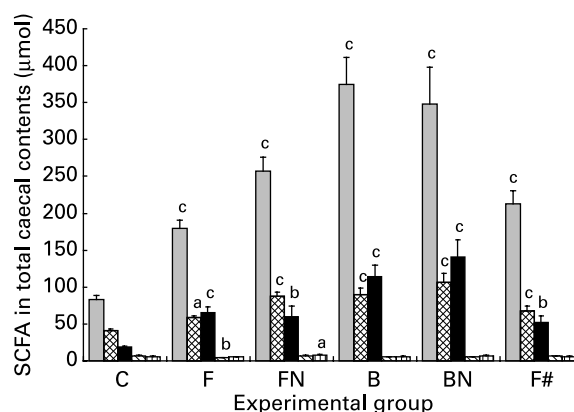


Fig. 4. Individual SCFA in the whole-caecal contents (μ mol) of rats fed the control diet C or the oat-based diets F, FN, B, BN and F# for 6 weeks. Values are means of ten rats with their standard errors shown by vertical bars. Mean values were significantly different from those of the control group: ^a*P*<0.05, ^b*P*<0.005, ^c*P*<0.001. □, Acetate; ▨, Propionate; ■, Butyrate; ▩, iso-Valerate; ▤, n-Valerate. For details of diets, see p. 1013 and Table 1.

which high concentrations of SCFA were also present. Therefore, besides different rates of interaction between DF components in the diet and BA, and different transport rates of BA into the caecum, the fermentation of DF seems to play an important role in the formation of secondary BA. The greatest amount of secondary BA was found in group F. In whole contents of proximal colon, a lower concentration of primary and secondary BA appeared in groups fed the Novelose-containing diets. The proportion of primary and secondary BA in faeces at week 6 is given in Fig. 7. In all test groups, significantly more primary and fewer secondary BA (*P*<0.001) were found than in the control group. Thus, the proportion of primary BA was 36.7% in the controls and 56.5% in group BN.

A greater concentration of NS was found in the whole-caecal contents of rats fed the oat-based diets compared with controls (*P*<0.001): control group, 18.47 (SEM 1.41) μ mol; group F, 26.99 (SEM 0.46) μ mol; group FN, 40.50 (SEM 2.28) μ mol; group B, 29.06 (SEM 1.09) μ mol; group BN, 33.15 (SEM 1.18) μ mol; group F#, 24.64 (SEM 0.60) μ mol. Obviously, Novelose had a high impact on the transport of NS into the caecum.

Table 5. Concentration of individual and total bile acids (μ mol/g DM) in the faeces of rats fed control or oat-based diets for 6 weeks

(Values are means of ten rats with their standard errors)

Bile acid	Control		Flour		Flour/Novelose		Bran		Bran/Novelose		Flour (autoclaved)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
CA	0.938	0.022	1.372 ^c	0.024	1.556 ^c	0.044	1.559 ^c	0.036	1.841 ^c	0.038	1.004 ^c	0.026
DCA	1.952	0.021	2.110 ^a	0.045	1.945	0.049	2.270 ^c	0.059	2.225 ^c	0.040	1.831	0.027
KDCA	0.267	0.011	0.385 ^c	0.022	0.468 ^c	0.023	0.527 ^c	0.016	0.556 ^c	0.024	0.357 ^c	0.013
KLCA	0.802	0.012	0.903 ^b	0.024	0.717 ^a	0.020	0.926 ^c	0.024	0.754	0.023	0.688 ^c	0.016
CDCA	0.313	0.009	0.700 ^c	0.021	0.701 ^c	0.012	0.639 ^c	0.028	0.865 ^c	0.017	0.565 ^c	0.017
LCA	2.555	0.044	2.593	0.049	2.245 ^c	0.041	2.891 ^c	0.050	2.453	0.046	2.323 ^b	0.050
α MCA	1.615	0.032	2.312 ^c	0.037	2.522 ^c	0.049	2.775 ^c	0.045	2.938 ^c	0.046	2.142 ^c	0.043
β MCA	1.315	0.037	2.073 ^c	0.038	1.561 ^b	0.043	2.208 ^c	0.047	2.180 ^c	0.062	1.820 ^c	0.044
HDCA	2.419	0.038	1.797 ^c	0.045	1.667 ^c	0.043	1.834 ^c	0.034	1.358 ^c	0.047	1.469 ^c	0.039
UDCA	0.208	0.013	0.461 ^c	0.017	0.494 ^c	0.020	0.297 ^b	0.017	0.450 ^c	0.018	0.484 ^c	0.023
Total BA	12.384	0.082	14.706 ^c	0.109	13.875 ^c	0.140	15.925 ^c	0.104	15.618 ^c	0.142	12.683	0.093

CA, cholic acid; DCA, deoxycholic acid; KDCA, 7-ketodeoxycholic acid; KLCA, 12-ketodeoxycholic acid; CDCA, chenodeoxycholic acid; LCA, lithocholic acid; MCA, muricholic acid; HDCA, hyodeoxycholic acid; UDCA, ursodeoxycholic acid. BA, bile acid.

Mean values were significantly different from those of the control group: ^a*P*<0.05, ^b*P*<0.005, ^c*P*<0.001.

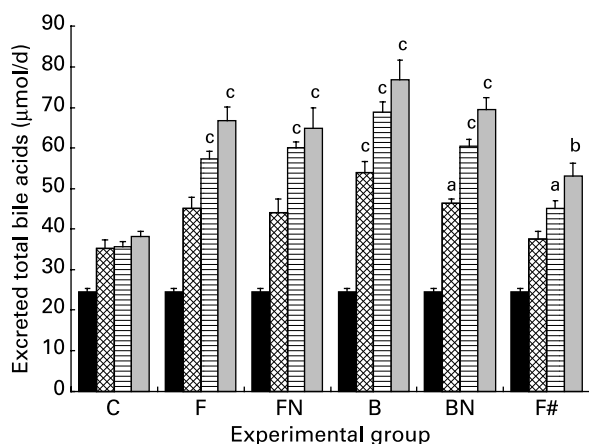


Fig. 5. Excretion of total bile acids ($\mu\text{mol/d}$) of rats fed the control diet C or the oat-based diets F, FN, B, BN and F# during the experiment. Values are means of ten rats with their standard errors shown by vertical bars. Mean values were significantly different from those of the control group: ^a $P < 0.05$, ^b $P < 0.005$, ^c $P < 0.001$. ■, week 0; ▨, week 2; ▩, week 4; □, week 6. For details of diets, see p. 1013 and Table 1.

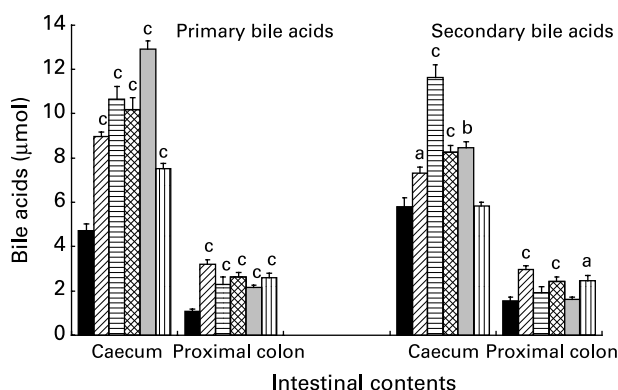


Fig. 6. Primary and secondary bile acids in whole-caecal and proximal colonic contents (μmol) of rats fed the control diet C or the oat-based diets F, FN, B, BN and F# for 6 weeks. Values are means of ten rats with their standard errors shown by vertical bars. Mean values were significantly different from those of the control group: ^a $P < 0.05$, ^b $P < 0.005$, ^c $P < 0.001$. ■, C; ▨, F; ▩, FN; ▧, B; □, BN; ▩, F#. For details of diets, see p. 1013 and Table 1.

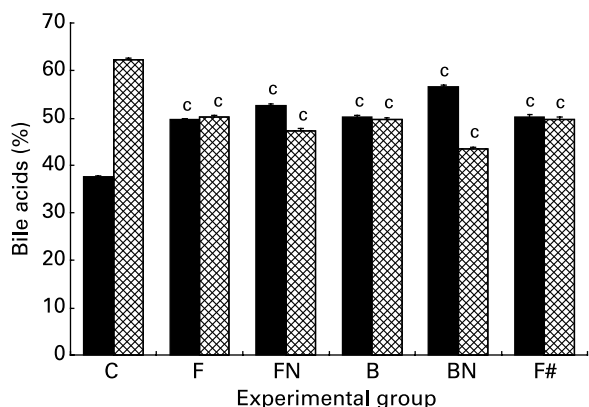


Fig. 7. Proportion of primary and secondary bile acids in the faeces (%) of rats fed the control diet C or the oat-based diets F, FN, B, BN and F# for 6 weeks. Values are means of ten rats with their standard errors shown by vertical bars. Mean values were significantly different from those of the control group: ^c $P < 0.001$. ■, Primary bile acids; ▨, Secondary bile acids. For details of diets, see p. 1013 and Table 1.

Approximately 74% of the NS were cholesterol and 25% were coprostanol. Coprostanone was present only in very low amounts. In the contents of the proximal colon, the following total NS concentrations were found: control group, 3.98 (SEM 0.43) μmol ; group F, 10.83 (SEM 0.56) μmol ; group FN, 7.34 (SEM 0.98) μmol ; group B, 9.38 (SEM 0.74) μmol ; group BN, 6.89 (SEM 0.41) μmol ; group F#, 8.38 (SEM 0.83) μmol ($P < 0.05$ or 0.001 v. control). The highest NS contents were present in the groups fed the diets with extruded flour and bran without Novelose. The NS in the colonic contents consisted of approximately 41% cholesterol, 56% coprostanol and 3% coprostanone.

Whereas the amount and composition of excreted NS were hardly different in the control group throughout the experiment, the concentration of individual NS increased from week 0 to week 6 in groups fed the oat-based diets (with the exception of group F#). The concentration of individual NS at week 6 is shown in Fig. 8. Most NS were excreted in groups fed the bran-containing diets. The proportion of cholesterol was only 33.7% in the control group and 29.8–33.1% in the test groups in the faeces at week 6. Small amounts of cholestanone appeared in some groups. The following concentrations of total NS/d were excreted at week 6: control group, 63.62 (SEM 1.58) μmol ; group F, 119.70 (SEM 7.15) μmol ; group FN, 119.90 (SEM 9.35) μmol ; group B, 143.97 (SEM 9.02) μmol ; group BN, 125.94 (SEM 3.57) μmol ; group F#, 88.54 (SEM 5.55) μmol ($P < 0.001$ v. control; for group F#, $P = 0.002$).

The excretion of total steroids (the sum of BA and NS) increased in all groups during the experiment (Fig. 9). The highest steroid excretion was found in group B. The addition of Novelose (group BN) resulted in a lower total amount of excreted steroids. There were no differences in steroid excretion between the groups whose diets contained extruded oat flour. Furthermore, small amounts of phytosterols were present in the faeces (data not shown).

Discussion

Cereals and cereal products, particularly from whole grains, are the most important source of DF in the Western diet. The amount and composition of DF differ between cereals and cereal cultivars and depend on the technology used during preparation of the cereal products. Despite oat products being well accepted, less than 10% of total oat production is used for human nutrition.

Starch is usually not eaten in its native state. During cooking processes, starch is gelatinised, with the formation of a starchy paste. With the exception of its resistant fraction, starch can be hydrolysed by enzymes in the small intestine in this state. Extrusion is suitable for the preparation of DF-rich products (Lue *et al.* 1991; Wang & Klopfenstein, 1993; Huth *et al.* 2000). Preparing starchy products that can be eaten directly, for example ready-to-eat products, snack foods or breakfast cereals, is a technological process (Colonna *et al.* 1989). Because of the mechanical and thermal energy input, the morphological and molecular structure of starch is changed. The elevated pressure and high temperature employed during the extrusion process, as well as variables of the ingredients, alter the physical, chemical, functional, sensory and nutritional properties of the extruded products (Asp & Björk, 1989; Liu *et al.* 2000). Thus, extrusion results in increased alkaline viscosity and soluble DF content, including that of soluble β -glucan (Berglund *et al.* 1994).

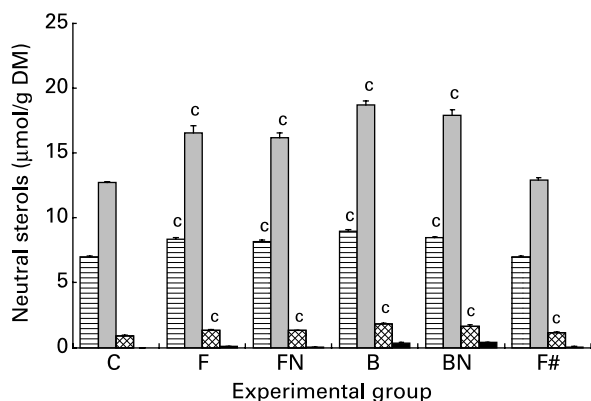


Fig. 8. Excretion of individual neutral sterols ($\mu\text{mol/g DM}$) of rats fed the control diet C or the oat-based diets F, FN, B, BN and F# for 6 weeks. Values are means of ten rats with their standard errors shown by vertical bars. Mean values were significantly different from those of the control group: $^{\circ}P < 0.001$. \square , Cholesterol; \square , Coprostanol; \boxtimes , Coprostanone; \blacksquare , Cholestanone. For details of diets, see p. 1013 and Table 1.

There is some information on the nutritional properties of cereal extrudates (Asp & Björk, 1989; Kahlon *et al.* 1998). Chang *et al.* (2002) found that extruded cassava starch products blended with either the RS preparation Novelose or oat fibre reduced LDL- and total cholesterol as well as triacylglycerols in hypercholesterolaemic hamsters. Dongowski *et al.* (2002a, 2003) showed that barley extrudates had beneficial nutritional effects in rats.

By the combined extrusion of cereals and Novelose, products with improved textural properties and higher DF contents, including increased RS concentrations, can be prepared (Anon., 1996). Such products, rich in different types of DF and more or less belonging to the whole-grain type, are multifunctional foods with a high nutritional relevance. Besides RS, β -glucan was the most important DF component in the oat-based products used. Delaney *et al.* (2003a) showed that the administration of high β -glucan concentrations (up to 7%) in the diet had no toxic effects on rats. β -Glucan solutions had a complex rheological behaviour depending on the source material, the technological pre-treatments (Zhang *et al.* 1998) and the concentration used.

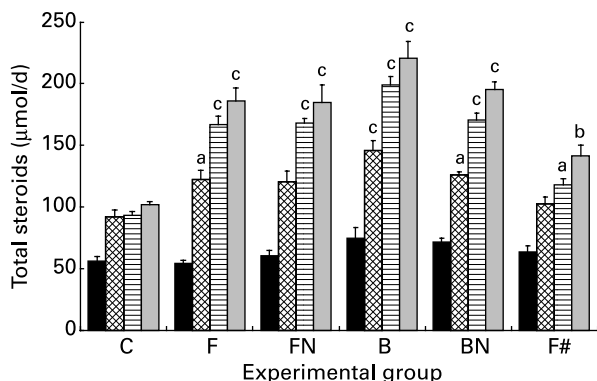


Fig. 9. Excretion of total steroids ($\mu\text{mol/d}$) of rats fed the control diet C or the oat-based diets F, FN, B, BN and F# during the experiment. Values are means of ten rats with their standard errors shown by vertical bars. Mean values were significantly different from those of the control group: $^aP < 0.05$, $^bP < 0.005$, $^{\circ}P < 0.001$. \blacksquare , week 0; \boxtimes , week 2; \square , week 4; \square , week 6. For details of diets, see p. 1013 and Table 1.

The rheological behaviour of β -glucan isolated from the DF-rich oat products was recently evaluated using oscillatory and shear measurements (Dongowski *et al.* 2005). It was found that β -glucan extracted from extruded flour and from bran had a dominant viscous behaviour, whereas the β -glucan from autoclaved flour demonstrated an elastic behaviour. The β -glucan solutions were structurally viscous, non-Newtonian solutions with a rheo-stable behaviour. These rheological properties may influence the flow, diffusion or transport behaviour during digestion processes in the small intestine.

We propose that it would be advantageous to apply combinations of cereals (whole-grain flour, bran) and RS in preventive nutrition. RS may differ widely in their characteristics, there being four different types: (1) physically inaccessible starch of whole or partly milled grains; (2) native granular starches; (3) retrograded starches; (4) chemically modified starches. Type 3, the most important RS, can be produced from gelatinised amylose or de-branched starches by controlled re-crystallisation (Asp *et al.* 1996). Schmiedl *et al.* (2000) showed that a degree of polymerisation of between 20 and 35 glucose units is optimal for a heat-stable RS type 3, giving a high proportion of butyrate during its fermentation. Novelose 330, used in this study, contained approximately 45% v/v RS type 3.

There was no difference in food intake or weight gain among the groups in the present experiment, but water intake was higher in the groups fed the oat-based diets. In a previous study, a higher food intake was observed in rats fed barley-based diets at weeks 5 and 6 (Dongowski *et al.* 2002a).

Diet influences the balance of intestinal microflora in a complex manner. As a result of DF fermentation, the qualitative and quantitative composition of the microflora (germ numbers, bacterial species) may be changed. This effect was shown for prebiotics, which are mainly non-digestible but fermentable oligosaccharides (Blaut, 2002). In the present study, the intestinal microflora were influenced to only a small degree by the diets used. Feeding the oat-based diets resulted especially in a decrease in the number of coliforms and an increase in bifidobacteria. It should be mentioned that the application of DF-rich barley diets resulted in higher count numbers of *Lactobacillus* (Dongowski *et al.* 2002a).

The effects of DF on the intestinal microbiota depend first on the structure and solubility of the DF components and second on their concentration and on the adaptation as well as the application period. Thus, several *Bacteroides* and *Escherichia coli* strains were involved in the degradation of pectin (Jensen & Canale-Parola, 1986; Tierny *et al.* 1994; Dongowski *et al.* 2000). A stimulation of beneficial bacteria (*Bifidobacterium*, *Lactobacillus*) and a partial inhibition of the growth of harmful bacteria (*E. coli*) were found if inulin, non-digestible oligosaccharides or other prebiotics were applied (Gibson & Roberfroid, 1995; Gibson, 1998; Kruse *et al.* 1999). Despite the fact that RS is not considered to be a prebiotic, it still exhibits prebiotic properties. This effect was also observed in the present experiment.

The presence of viscous DF such as β -glucan in the oat-based diets, and therefore also in the gut, resulted in higher masses of the caecum and colon walls in most of the test groups. This effect was accompanied by a greater thickness of the walls and changes in the proliferative behaviour of the mucosa (data not shown). Furthermore, the amounts of wet content in the lower intestine were higher in the experimental groups. Such effects were also found in studies with barley-based diets

(Dongowski *et al.* 2002a) or pectin (Schmehl *et al.* 1997). Motility was increased because much more material must be transported through the caecum and colon in the presence of DF-rich diets.

Several studies have shown that DF can be fermented *in vitro* and *in vivo*. Thus, Drzikova *et al.* (2005a) found that oat-based extrudates (very similar to the oat products used in the present study) were fermented *in vitro* with human faecal flora under the formation of high concentrations of SCFA. More SCFA were produced when the extrudates contained more oat bran, soluble and insoluble DF and β -glucan.

In vivo, DF are partly or completely fermented by the intestinal microflora, depending on their structural and physicochemical parameters (Bourquin *et al.* 1996; Wood *et al.* 2002). Before their fermentation, the poly- and oligosaccharides must be first split into their monosaccharide units by bacterial enzymes. The monosaccharides formed are then further enzymatically decomposed via the Emden–Meyerhof–Parnas and pentose phosphate pathways (Macfarlane & Gibson, 1996). Some SCFA, such as *n*- and *iso*-valeric acid, are formed by the deamination of amino acids as a result of protein fermentation (De Schrijver, 1996). High concentrations of SCFA, particularly butyrate, in the lower parts of the intestinal tract are important for a healthy colonic mucosa. Butyrate, the physiologically most important SCFA, is the major energy source for the colonic epithelium. It regulates cell growth and differentiation as well as playing a role in protection against colon cancer (Topping & Clifton, 2001; Smith *et al.* 1998; Jacobasch & Dongowski, 2000). Propionate has been proposed as an inhibitor of hepatic cholesterol synthesis (Anderson *et al.* 1990).

β -Glucan or RS can be fermented relatively rapidly up to 100%, whereas cellulose is usually incompletely or not fermented. Both groups of DF are important for healthy nutrition. The unfermented DF can increase colonic luminal content and lower the transit time through the large intestine, as well as binding water and 'diluting' carcinogens and toxic substances. On the other hand, fermentable DF are an essential source for the formation of SCFA.

We found higher total concentrations of SCFA and higher proportions of butyrate in the caecal contents of rats given the oat-based diets compared with the control group. SCFA formation was influenced by DF composition, DF concentration in the diets and the technological pre-treatment of the oat products.

As an essential component of the mixed micelles, BA are necessary for lipid digestion in the small intestine. They are normally almost completely absorbed in the ileum by active and passive mechanisms, and are transported to the liver via the enterohepatic circulation (Hofmann, 1994). Several DF can interact with BA under the conditions found in the small intestine (Hoagland & Pfeffer, 1987; Dongowski, 1995) or may disturb fat digestion. These effects result in a lower reabsorption of BA, in their greater transport towards the large intestine (Zhang *et al.* 1992), in their increased microbial conversion (such as deconjugation and dehydroxylation) and finally in their higher excretion (Zhang *et al.* 1993; Marlett *et al.* 1994; Lia *et al.* 1997; Dongowski *et al.* 2003). Therefore, the increased excretion of BA requires an increased hepatic synthesis of BA from cholesterol in the blood (Andersson *et al.* 2002). This is a major hypocholesterolaemic pathway, occurring especially in hypercholesterolaemic individuals or animals (Braaten *et al.* 1994; Garcia-Diez *et al.* 1996; Chang *et al.* 2002). Hydrophobic

effects seem to play a role in the interactions between BA and DF (Dongowski, 1995). Furthermore, these interactions may be influenced by the molecular weight and viscosity of isolated DF preparations or by the surface, particle size, composition, botanical source and pre-treatment of DF preparations with cell wall structures (Mongeau & Brassard, 1982; Huang & Dural, 1995; Dongowski & Ehwald, 1999; Kahlon & Woodruff, 2003; Kotcharian *et al.* 2004).

A strong interaction was found between digested oat-based extrudates and BA *in vitro*. The binding of BA increased with increasing proportions of oat bran, total DF and insoluble DF, as well as with increasing contents of β -glucan in the extrudates. Dihydroxy BA were bound more strongly to the extrudates than were trihydroxy BA. These interactions were greater at pH 5.0 than at pH 6.5 (Drzikova *et al.* 2005a). Likewise, Kahlon & Woodruff (2003) found an interaction between BA and cereal brans.

In the intestinal contents of rats fed the oat-based diets, more total BA were present compared with the control group. Likewise, BA excretion was higher in these groups, an exception being the group fed the autoclaved oat flour. Oat bran was most effective in BA transport into the lower parts of intestinal tract and excretion. The molecular weight of DF such as β -glucan and the degree of destruction of the plant cell walls seem to play a role in interactions with BA. Such effects may occur during technological treatments (cooking, extrusion, autoclaving, etc.) or during passage through the upper gastrointestinal tract (Sundberg *et al.* 1996; Johansen *et al.* 1997; Robertson *et al.* 1997).

As a result of the lower pH of the intestinal contents in groups fed the oat-based products, more primary BA and a lower proportion of secondary BA were found. The concentration of secondary BA was, however, relatively high in the test groups. Secondary BA are formed from primary BA by enzymatic 7α -dehydroxylation of the steroid nucleus. Such activity was found in *Eubacterium* and *Clostridium*. Other enzymatic actions may lead to the insertion of OH groups at C atom 6, the formation of β -OH groups from α -OH groups, or the formation of keto groups from OH groups on the steroid nucleus (Baron & Hylemon, 2000). The involvement of secondary BA in colon carcinogenesis is not yet clear (Hofstad *et al.* 1998; Roy *et al.* 1999). Certain secondary BA (e.g. deoxycholic acid, lithocholic acid) may be cytotoxic and may promote colon carcinogenesis when present in abnormally high concentrations (Owen, 1997). Cytotoxicity depends on the hydrophobicity of the BA (Hofmann, 1999). The best strategy to overcome the problematical effects of BA in the colon is a DF-rich diet. The following effects may occur in the presence of DF: decrease of transit time; dilution of BA; inclusion of BA in the swollen, unfermented DF fraction; lowering of the proportion of secondary BA; improvement in health of the colon mucosa by higher concentrations of SCFA; a healthy microflora. Thus, butyrate and deoxycholic acid appear to interact in a complex and antagonistic manner to selectively modulate crypt base and surface proliferation in the rat colon (Velázquez *et al.* 1997). It was also shown in the present study that intestinal butyrate was increased in groups fed the oat-based diets.

Likewise, more NS were present in the caecal and colonic contents, as well as in the faeces, of the test groups. A higher excretion rate of NS is connected with a lower absorption of cholesterol. Coprostanol formed from cholesterol by intestinal microflora (Baron & Hylemon, 2000) was the main excreted NS. Similar effects were found in rats fed DF-rich, barley-based diets (Dongowski *et al.* 2003). Furthermore, more total steroids

were excreted in almost all groups given extruded oat-based diets or oat bran.

Despite a higher excretion of BA, the effects of the oat-based diets on serum lipid concentration were low because the animals had normal plasma lipid concentrations.

Generally speaking, RS has a reduced caloric content and lowers the postprandial glucose and insulin responses (Granfeldt *et al.* 1995). As has also been shown in the present study, the most important effect of RS consumption is the increased intestinal SCFA concentration, including a high proportion of butyrate. In several studies, it was found that the application of RS could result in a lower excretion of BA and NS (Hylla *et al.* 1998; Langkilde *et al.* 1998, 2002). It therefore seems possible that changing the cereal–RS relations can modulate the transport of BA towards the lower parts of the intestinal tract, their partial conversion to secondary BA and their excretion. In addition, van Munster *et al.* (1994) found that faecal water cytotoxicity and colonocyte proliferation were lowered by RS diets. Jacobasch *et al.* (1999) showed effects of RS on inflammatory bowel diseases. Some studies indicate that RS may be protective against tumour development, whereas no protection has been found in other studies (Martin *et al.* 2000; Topping & Clifton, 2001).

There is some confusion in the literature regarding the relationship between DF intake and cancer, especially from the data of several large cohort studies. Thus, Fuchs *et al.* (1999) and other authors (e.g. data from the Nurses Health Study) found no relationship between DF intake and colorectal cancer. In contrast to these epidemiological evaluations, positive effects of DF on inflammatory bowel diseases and colorectal cancer have been shown by most experimental studies (Kritchevsky & Bonfield, 1995; Mälkki & Cummings, 1996; Hartemink, 1997; Spiller, 2001; van der Kamp *et al.* 2004). Recently, Bingham *et al.* (2003) found, in the European Prospective Investigation into Cancer and Nutrition cohort study, that an approximate doubling of total DF intake from food could reduce the risk of colorectal cancer by 40%. Whereas in cohort studies DF content is calculated from data reported in questionnaires, well-defined and exactly determined DF components are applied in most experimental studies. As shown in the present study, individual DF components or groups, such as β -glucan and RS or the grown cell wall structure, differ in their structure and functional properties and may have special effects in the gastrointestinal tract.

One question that can be raised relates to whether the concentration of oat products used in the test diets of the present study was too high. The test diets, however, contained all nutrients in their recommended concentrations. We replaced a great part of the wheat starch, used in the control diet, by the oat products, consisting likewise mainly of starch. Naturally, the test diets were lower in energy and higher in DF than the control diet. In a former study, we used test diets consisting of 500 g/kg barley products (Dongowski *et al.* 2002a, 2003). There were no differences in weight gain between all groups and no illness seen in the rats, either in the experiment with the barley-containing diet or with the oat-containing diets in the present experiment. On the other hand, a long-term application of such high concentrations of oat products is not practicable in human nutrition. In pilot studies, subjects were given 100 g of an oat-based or a barley-based DF-rich extrudate per day for 4 weeks in addition to their habitual diet (Drzikova *et al.* 2005b; G. Dongowski *et al.* unpublished results). Principally, it appears that there is no problem consuming this amount of extrudate for a longer period.

Using the definition of DF given by the Food and Nutrition Board of the National Academy of Sciences of the USA (Anon., 2002), the RS-containing products investigated in the present study can be described as functional fibre.

In conclusion, the application of the DF-rich, oat-based diets had a variety of beneficial physiological and protective effects in rats depending on the composition and amount of the DF, their technological pre-treatment and their functional properties. The major effects were connected with the fermentation of DF components and the higher formation and absorption of SCFA, as well as with the higher excretion of steroids. Further studies are requested in man to prove whether a regular consumption of such oat-based products is helpful in preventing inflammatory bowel diseases and colon cancer, as well as against hypercholesterolaemia.

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