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Steroids in the intestinal tract of rats are affected by dietary-fibre-rich barley-based diets

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The aim of the present study was to investigate the influence of dietary-fibre (DF)-rich barley-based diets on bile acids (BA) and neutral sterols (NS) in the intestinal tract of rats. For this purpose, young male Wistar rats (*n* 50; ten per group) weighing about 67 g were fed either a barley-free diet (control group) or diets containing 500 g barley meal extrudates/kg or a barley meal–Novelose mixture (groups A–D) for 6 weeks. These barley products contained 7–24 g resistant starch/100 g and 7–12 g (1 → 3),(1 → 4)-β-glucan/100 g. More steroids were transported towards the lower parts of the intestinal tract when higher concentrations of macromolecular DF were present in the diets ($P<0{\cdot}001$). Tauroconjugated and primary BA dominated in the contents of the small intestine. Intense enzymic conversion of BA occurred in the caecum and colon. The fermentation of DF affected indirectly the amount of formed secondary BA. The main BA present in the caecal contents were muricholic acids, hyodeoxycholic acid and cholic acid. The BA spectrum in the colonic contents was different from that in the caecum. A higher concentration of NS appeared in the intestinal contents of the groups fed the barley-based diets than in the controls ($P<0{\cdot}005$). The microbial conversion of cholesterol to coprostanol, cholestanone and coprostanone was influenced by the amount and composition of the DF in the gut. DF in the diet may affect the concentration and spectrum of steroids in the intestinal tract. The results are relevant for the discussion of mechanisms behind the cholesterol-lowering effects of DF.

Barley: β-Glucan: Resistant starch: Steroids

A high intake of dietary fibre (DF) is associated with several preventive–medical effects in man and animals. DF have been recognised as a necessary nutrient group in human nutrition. But the recommended daily DF intake of at least 30 g for adults (Deutsche Gesellschaft für Ernährung, 2000) is achieved in most of the Western countries. Besides fruits and vegetables, wholegrain products are the most important source of DF. Grains are rich in different types of DF, most DF being polysaccharides; for example, β-glucans, arabinoxylans, cellulose and hemicelluloses. Furthermore, non-digestible oligosaccharides are also present in cereals. The amount and composition of DF differ between the cereals and depend on the technology applied during the preparation of cereal products.

The polysaccharide (1 → 3),(1 → 4)-β-glucan (from here on called β-glucan), present in the cell walls of barley and oats, is not only an important DF component in food but also in nutrition because of its rheological behaviour, viscosity and functional properties in the gastrointestinal tract (Lund *et al.* 1989; Doublier & Wood, 1995; Tejinder *et al.* 2000). The feeding of β-glucan-rich diets resulted in several beneficial physiological effects (Mälkki & Virtanen, 2001). Thus, cholesterol and lipoprotein fractions were affected by β-glucan intake in man (McIntosh *et al.* 1991; Braaten *et al.* 1994) and animals (Kahlon *et al.* 1993; Hecker *et al.* 1998; Kalra & Jood, 2001) in some studies. Recently, the effects of β-glucan on human serum lipoprotein were critically reviewed from the literature data (Kerckhoffs *et al.* 2002). The cholesterol-lowering potency of β-glucan was approximately identical in hamsters independent of its origin being from either oats or barley (Delaney *et al.* 2003).

The plasma cholesterol-lowering properties of DF such as β-glucan, psyllium (Romero *et al.* 2002), guar gum (Fernandez *et al.* 1995; Seal & Mathers, 2001), pectin (Fernandez *et al.* 1994), algal polysaccharides (Dvir *et al.* 2000; Seal & Mathers, 2001) or special DF sources such as rhubarb stalk fibre (Goel *et al.* 1999) seem to be mostly related to their macromolecular state. The plasma cholesterol-lowering effects are found especially in hypercholesterolaemic human subjects or animals, and are connected directly or indirectly with the metabolism of bile acids (BA) and neutral sterols (NS) in the intestinal tract and liver. Thus, Terpstra *et al.* (2000)

Abbreviations: BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; DF, dietary fibre; HDCA, hyodeoxycholic acid; LCA, lithocholic acid; MCA, muricholic acid; NS, neutral sterol; RS, resistant starch; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid.

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found that feeding rats psyllium-containing diets resulted in the lowering of plasma cholesterol concentration and in an increase in the faecal excretion of BA. Besides greater BA and total steroid excretion, psyllium also increased the activities of 7α-hydroxylase in rats (Buhman *et al.* 1998). Demigne *et al.* (1998) showed that guaran enhanced steroid excretion and changed enterohepatic cycling in rats. But also some oligomeric DF such as inulin were able to diminish plasma total cholesterol, VLDL-cholesterol and triacylglycerol concentrations as well as to alter the BA profile of gallbladder bile in hamsters (Trautwein *et al.* 1998).

In the last 20 years, it has been shown that a part of starch can be converted into resistant starch (RS) by special technological treatments. RS is defined as the starch or starch degradation products which are not absorbed in the small intestine of healthy individuals (Berry, 1986; Asp *et al.* 1996). RS is a major substrate for colonic fermentation and a good source of butyrate. In a previous study it was found that amylose can be partly converted into RS by extrusion under optimal conditions followed by freeze-storage whereas the macromolecular state of β-glucan was preserved. Such extruded products interacted *in vitro* with glycoconjugated BA. During their *in vitro* fermentation with human faecal flora, the production of short-chain fatty acids and the molar portion of butyrate were more strongly increased compared with barley meal (Huth *et al.* 2000). Vanhoof & De Schrijver (1998) found that the consumption of raw or high-amylose maize starch (both RS) resulted in a decrease in total and esterified cholesterol levels of liver and plasma as well as in a tendency to produce higher faecal coprostanol and total BA excretion in rats.

Besides starch and the DF components, several other components with nutritional relevance are present in barley: proteins (8–15%); lipids (2–3%); minerals; small quantities of vitamins; etc. Tocotrienols and α-linolenic acid present in barley oil were found to show cholesterol-lowering activities (McIntosh *et al.* 1995; Jadhav *et al.* 1998). The feeding of 30 g barley-bran flour or 3 g barley oil in the daily diet lowered total and LDL-cholesterol in hypercholesterolaemic human volunteers (Lupton *et al.* 1994). In several studies, it was shown that phytosterols also, as the counterparts of cholesterol in man and animals, are able to reduce plasma lipids and to increase cholesterol excretion (Moghadasian, 2000; Kerckhoffs *et al.* 2002; Moreau *et al.* 2002; Trautwein *et al.* 2002). Compared with maize, the phytosterol content of barley is relatively low (Piironen *et al.* 2000). It is now well accepted that combinations of groups of different compounds present in wholegrain products, fruits and/or vegetables are often more effective from the nutritional viewpoint than their isolated consumption.

There is very little information on the effects of barley-rich diets on the steroid pattern in the intestinal tract of man and animals. Thus, Lia *et al.* (1995) found a greater excretion of BA and cholesterol in ileostomy patients when they were given barley diets.

In a complex study (Dongowski *et al.* 2002), the physiological effects of DF-rich barley-containing diets (particularly extrudates) from the wholegrain type were evaluated in male Wistar rats. The diets that were fed differed in their concentrations of total DF and of individual DF (for example, β-glucan, RS). The aim of that study was to investigate if barley-rich diets with an optimised DF composition would cause beneficial direct or indirect physiological effects in the intestinal tract. It was shown that feeding rats the diets containing 50 g barley products/100 g for 6 weeks resulted in increased masses of the caecum and colon as well as in higher weights of the intestinal contents, and in greater amounts of excreted steroids compared with those rats fed with the barley-free control diet (Dongowski *et al.* 2002). There are only a few reports on the amount of steroids found in the intestinal tract during the feeding of barley-containing diets (Gallaher *et al.* 1992; Lia *et al.* 1995).

The present study was undertaken to assess the effects of DF-rich barley-based diets on the concentration and composition of BA and NS and on their conversions during their passage through the intestinal tract of male Wistar rats. Special focus was concentrated on the effects of β-glucan and RS-rich diets based on barley products of the wholegrain type with regard to effects on steroid metabolism.

Materials and methods

Barley products and barley-based diets

Source materials for the preparation of barley products A–D. The barley mutant '(HiAmi × Cheri) × Cheri' containing (per 100 g) 6·5 g β-glucan, 56·9 g starch and 33·8 g amylose/100 g starch (from here on named 'HiAmi') was supplied by the Institute for Stress Physiology and Quality of Raw Materials, Groß Lüsewitz, and Saatzucht Dr H. C. Carsten, Bad Schwartau, Germany. The waxy barley variety 'Prowashonupana' containing (per 100 g) 17·6 g β-glucan, 30·1 g starch, 5 g amylose/100 g starch and 29·8 g DF was supplied by ConAgra, Omaha, NE, USA. Novelose 330 with approximately 30 g RS type III/100 g was supplied by National Starch & Chemical GmbH, Hamburg, Germany. Amylose from maize was supplied by Sigma, Deisenhofen, Germany.

Barley products A ('HiAmi'), B ('HiAmi' – 'Prowashonupana'; 50:50, w/w) and D ('Prowashonupana' – Amylose; 60:40, w/w) were prepared by extrusion using a feed moisture content of 20%, a mass temperature of 150°C, a speed of 350 rpm, and a diameter of the dies of 2 × 3·5 mm in the twin-screw extruder APV 50 (Baker-Perkins, Peterborough, Cambs, UK). All extrudates were stored for 3 d at −18°C before use. Barley product C was prepared by mixing 'Prowashonupana' and Novelose (50:50, w/w).

The composition of the diets (prepared as pellets in the German Institute of Human Nutrition, Potsdam-Rehbrücke) is shown in Table 1. Diets A–D containing 50 g of the barley products A–D/100 g were prepared by partial replacement of the wheat starch used in the control diet.

Analytical methods

β-Glucan was determined after hydrolysis with lichenase and β-glucosidase (Megazyme International, Bray, Republic

Table 1. Composition of the control diet and the barley-based diets A–D

Diet...	Control diet	Diet A	Diet B	Diet C	Diet D
Diet ingredient (g/kg)					
Casein*	200	200	200	200	200
Wheat starch†	630	130	130	130	130
Barley sample	0	500	500	500	500
Sunflower-seed oil‡	50	50	50	50	50
Microcrystalline cellulose§	50	50	50	50	50
Mineral mixture‖	50	50	50	50	50
Vitamin mixture¶	20	20	20	20	20
Analysed composition (g/kg)**					
β-Glucan	0·3	38·4	60·3	42·0	67·8
Resistant starch	4·5	44·1	38·0	117·9	89·0
Soluble dietary fibre	< 1	21·4	59·2	28·8	47·7
Insoluble dietary fibre	48·0	99·9	112·9	165·8	133·1

* Dauermilchwerk Peiting GmbH, Landshut, Germany.
† Heller und Strauss, Berlin, Germany.
‡ Thomy GmbH, Karlsruhe, Germany.
§ Rettenmeier GmbH, Ellwangen, Germany.
‖ Altronim GmbH, Lage, Germany. The composition of the vitamin mixture was (mg/kg diet): retinyl palmitate, 7; cholecalciferol, 0·02; α-tocopheryl acetate, 240; menadione, 15; thiamin, 30; riboflavin, 30, pyridoxine, 22·5; cyanocobalamin, 0·05; niacin, 75; pantothenic acid, 75; folic acid, 15; biotin, 0·03; choline, 1500; *p*-aminobenzoic acid, 150; *myo*-inositol, 150.
¶ Altronim GmbH, Lage, Germany. The composition of the mineral mixture was (mg/kg diet): Ca, 9300; P, 7300; K, 7100; Na, 4400; Cl, 3600; S, 1700; Mg, 800; Fe, 200; Mn, 100; Zn, 30; Cu, 8; F, 4; I, 0·4; Se, 0·2; Co, 0·1.
** Mean values of at least duplicates; for β-glucan and resistant starch, n 4–6.

of Ireland) (McCleary & Mugford, 1997) as liberated glucose with the hexokinase–glucose-6-phosphate dehydrogenase kit from Boehringer (Mannheim, Germany).

The starch content was analysed enzymically using amyloglucosidase and the Boehringer glucose kit after extraction with 1 M-NaOH or dimethylsulfoxide–HCl. RS was measured *in vitro* according to the method of Berry (1986).

Total, insoluble, and soluble DF were analysed by the enzymic–gravimetric Association of Official Analytical Chemists method (Prosky *et al.* 1988).

Rat experiment

Male growing Wistar rats (Tierzucht Schönwalde GmbH, Schönwalde, Germany) weighing about 67 g were housed in pairs in temperature- and humidity-controlled cages (22 ± 2°C and 55 ± 5%) on a 12 h cycle of light (06.00–18.00 hours) and dark (18.00–06.00 hours). All animals were fed the control diet for 7 d after arrival (week 0). Then the rats were randomly divided into five groups of ten animals each and fed the control diet or the barley-based diets A–D for 6 weeks. The animals had free access to food and water. Several parameters (for example, food intake and weight gain) were determined weekly, some parameters in intervals of 2 weeks (for example, steroids in 24 h faeces and short-chain fatty acids in fresh faeces) and others in weeks 0 and 6 (microbial counts).

At the end of the experimental period (after 6 weeks of the diets), rats were killed by decapitation. The rats were given free access to food and water until 15 min before decapitation. The contents of the caeca and colons were skilfully prepared immediately and completely for steroid analysis. The small intestine was divided into equal lengths of upper and lower parts. For the following examinations, the contents of the lower parts exclusively were used. All data are expressed on a DM basis.

Extraction and determination of steroids

After the addition of internal standards (7α, 12 β-dihydroxy-5 β-cholanic acid for BA and 5-cholesten-3 β,25-diol or 5α-cholestan for NS), 50 mg of the freeze-dried intestinal content materials were treated for 1 h at 80°C under shaking with a mixture of 2·55 ml NaOH (0·5 M) and 23 ml ethanol (96%, v/v). After centrifugation (15 min at 4°C and 5000***g***), the supernatant fraction was concentrated to 3 ml in a Speed-Vac, mixed first with 7 ml NaOH (1 M) and 2 ml methanol and then with 10 ml hexane. The non-polar NS were separated from the BA by extraction with hexane (three times) by shaking and centrifugation (15 min at 4°C and 5000***g***). The purified NS-containing hexane phases were dried in a vacuum and dissolved in ethanol. After removal of the organic solvents in the Speed-Vac, the BA-containing phases were diluted with water and the BA were purified by solid-phase extraction on Bakerbond spe C_{18} columns in the BAKER spe-12G system (J.T. Baker, Gross Gerau, Germany).

The BA were estimated on a non-polar stationary phase (Nucleosil 100 Å; C_{18}; 5 μm; 250 × 4·6 mm) at 40°C in HPLC equipment from Gynkotek (Germering, Germany) after pre-column derivatisation with 4-bromomethyl-7-methoxycoumarin and fluorescence detection (excitation λ 320 nm; emission λ 385 nm) (Wang *et al.* 1990). Linear gradients consisting of acetonitrile (30–100%), methanol (40–0%) and water (30–0%) were applied.

The NS were determined on silica gel 60 F_{254} plates (Merck, Darmstadt, Germany) using the HPTLC system from Camag (Muttenz, Switzerland). The chromatograms were developed with ether–heptane (55:45, v/v). After dipping the plates for 3 s in a copper sulfate–phosphoric acid reagent followed by heating for 5 min at 180°C, the spots were measured at 405 nm using a TLC scanner II (Camag).

Reference steroids

α-, β- and ω-Muricholic acids (MCA), 7-ketodeoxycholic acid, 12-ketolithocholic acid and 5-cholesten-3 β,25-diol were obtained from Steraloids (Wilton, NH, USA). Taurodeoxycholic acid, cholic acid (CA), lithocholic acid (LCA), hyodeoxycholic acid (HDCA), 5α-cholestan-3-one (cholestanone) and 5α-cholestan were obtained from Sigma (St Louis, MO, USA). Chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), ursodeoxycholic acid, cholesterol and 5 β-cholestan-3-one (coprostanone) were obtained from Fluka (Neu-Ulm, Germany). Taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA) and 7α, 12 β-dihydroxy-5 β-cholanic acid were supplied by Calbiochem (La Jolla, California, USA). 5 β-Cholestan-3 β-ol (coprostanol) was obtained from Serva (Heidelberg, Germany).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences software SPSS 11.0 (SPSS Inc., Chicago, IL, USA). All values are given as arithmetical means and standard errors of the means. In some cases pooled standard errors of the means are given. Data were analysed by one-way ANOVA, and differences between the diet groups and the control group were evaluated by Dunnett's *t* test and Dunnett's T3-test for multiple *post hoc* comparisons. When variances were non-homogeneous, data were log-transformed before analyses. Differences with $P<0{\cdot}05$ were considered significant.

Ethical considerations

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 of Nutrition, Agriculture and Forestry (Germany) (permissions 48-3560-0/3).

Results

Barley samples and barley-based diets

Several DF components are present in barley meal. Barley cultivars with higher concentrations of β-glucan were selected for the present experiments. Huth *et al.* (2000) found that extrusion of barley meal under optimised conditions followed by a short-term freeze storage of the extrudates resulted in the generation of up to 7% RS. The prepared extrudates (barley products A, B and D) as well as the mixture prepared from the barley meal of 'Prowashonupana' and the commercial RS preparation Novelose 330 (barley product C) were relatively rich in β-glucan and RS. The concentrations of β-glucan found in barley products A–D were (g/100 g): 7·24 (SEM 0·01), 11·95 (SEM 0·04), 7·89 (SEM 0·05), and 10·57 (SEM 0·05), respectively (*n* 6). The concentrations of RS found in barley products A–D were (g/100 g): 6·56 (SEM 0·36), 6·74 (SEM 0·24), 24·25 (SEM 0·25), and 18·35 (SEM 0·16), respectively (*n* 6).

The compositions of the control diet and the barley-based diets A–D are summarised in Table 1. Diets A–D consisted of 50% of the barley samples. Therefore, they contained higher amounts of total DF, β-glucan and RS as well as less wheat starch than the control diet. Furthermore, all diets contained 5% of microcrystalline cellulose as a low-fermentable DF.

General effects of barley-based diets in rats

The barley-based and control diets were well tolerated by the rats. Food intake was significantly greater in the groups fed the barley-containing diets in the 5th and 6th week. Relatively small differences were measured in the weight gain between the rat groups. But in week 6, weights of the barley-fed rats were greater than in the control group. Food efficiency did not differ between all groups. In most of the caecal and colonic contents, significantly higher amounts of β-glucan and RS were found after 6 weeks in the rats fed the barley-based diets. At the end of the experiment, the numbers of coliforms and *Bacteroides* decreased in the barley groups B–D whereas higher numbers of *Lactobacillus* were found in groups B–D ($P<0{\cdot}05$). Further, concentrations of short-chain fatty acids including butyrate were higher in the caecal and colonic contents (after 6 weeks) as well as in faeces (between the 2nd and 6th week) in the rats fed the barley-based diets (Dongowski *et al.* 2002).

Amounts of intestinal contents

The following amounts of intestinal contents (wet-weight basis) were found in the second part of the small intestines of the rats after a feeding period of 6 weeks. The small-intestinal contents were (g): 1·42 (SEM 0·05) for the control group, 1·64 (SEM 0·12) for group A, 1·88 (SEM 0·13) for group B, 2·12 (SEM 0·08) for group C and 1·66 (SEM 0·14) for group D (*n* 10 per group). In groups B–D, the intestinal contents were greater than in the control group ($P<0{\cdot}001$). The wet caecal contents at the end of the experiment were (g): 5·38 (SEM 0·18) for the control group, 4·68 (SEM 0·22) for group A, 6·14 (SEM 0·25) for group B, 8·57 (SEM 0·29) for group C and 7·52 (SEM 0·45) for group D (*n* 10 per group). The caecal contents were greater in the groups fed diets C and D compared with the controls ($P\leq0{\cdot}001$). In all groups fed the barley-based diets, the amounts of colonic contents were greater than in the control group ($P<0{\cdot}001$). The following wet contents were found (g): 0·87 (SEM 0·05) for the control group, 1·63 (SEM 0·11) for group A, 1·63 (SEM 0·11) for group B, 1·47 (SEM 0·11) for group C and 1·71 (SEM 0·08) for group D (*n* 10) (Dongowski *et al.* 2002).

The steroid analyses are given per g DM. The total DM of the intestinal contents is summarised in Table 2. Compared with the control group, total DM content was greater in the small-intestinal contents (second part) of group C, in the caecal contents of groups B–D as well as in the colonic contents of all groups fed the barley-based diets.

Table 2. Total dry matter of intestinal contents of rats fed the control diet or the barley-based diets for 6 weeks*
(Mean values and standard errors of the means for ten rats per group)

	Control		Group A		Group B		Group C		Group D	
Intestinal contents	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Small intestine†	0·314[a]	0·011	0·345[a]	0·024	0·342[a]	0·024	0·464[b]	0·016	0·313[a]	0·027
Caecum	1·218[a]	0·042	1·116[a]	0·052	1·394[b]	0·056	1·731[c]	0·058	1·595[b]	0·096
Colon	0·301[a]	0·006	0·683[c]	0·048	0·699[c]	0·023	0·515[b]	0·019	0·678[c]	0·032

[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0·01$).
* For details of diets and procedures, see Table 1 and pp. 896–897.
† Second part of the small intestine.

Bile acids in intestinal contents

Feeding the barley-based diets resulted in greater amounts of BA in the second part of the small-intestinal contents of the rats compared with the controls ($P<0·001$), especially when β-glucan-rich diets were given (Table 3). High proportions of TCA were found in the small intestine (>50% of total BA) (Fig. 1). Further, taurodeoxycholic acid and TCDCA were present in greater amounts. Besides the tauroconjugated BA, only the MCA appeared in higher concentrations. Glycoconjugated BA which are dominant in human bile were not found in the small intestine of the rats. Free BA (i.e. not conjugated BA) and secondary BA (i.e. BA with no OH or keto group at the C-atom 7 of the steroid nucleus) were scarcely present in the ileum. Feeding the barley-based diets resulted in greater concentrations of most of the individual BA compared with the control group ($P<0·001$). Exceptions were the contents of both MCA in group A and of TCDCA in group C. Feeding the diets B and D, which contained the greatest amounts of β-glucan and soluble DF, resulted in the most effective transport of BA into the second part of the small intestine.

The total BA concentrations (per g DM) were greater in the caecal contents of the rat groups fed the barley-based diets compared with the controls ($P<0·001$); the greatest amounts of total BA were present in groups B and D (Table 3). The composition of the individual BA in the caecum was distinctly different from that found in the contents of the small intestine. Thus, the amounts of TCA and TCDCA were lower (Fig. 2), resulting in higher concentrations of the corresponding free BA (CA and CDCA). Further, more MCA and individual secondary BA (DCA, LCA, HDCA) were present in the caecum of all groups. In detail, 11·0–18·9% HDCA, 11·0–18·9% βMCA, 11·0–15·6% LCA, 10·4–15·3% αMCA, and 9·8–11·6% TCA were found in the caecal contents. Compared with the controls, more CA, αMCA, βMCA, ursodeoxycholic acid and DCA as well as less TCDCA were present in the caecal contents of all groups fed the barley-based diets ($P<0·001$). However, the picture was more complex in the case of some BA. Thus, concentrations of HDCA were only lower in groups B and D than in the control group ($P<0·002$).

In the colonic contents, more total BA (per g DM) were present in the groups fed the barley-based diets than in the controls ($P<0·001$) (Table 2). The greatest total concentrations of BA appeared in the rats fed the β-glucan-rich diets B and D. In these groups, high amounts of CA and αMCA were found whereas concentrations of individual tauroconjugated BA were low (Fig. 3). In detail, 18·5–20·2% αMCA, 12·8–18·3% HDCA, and 12·5–13·9% DCA were present in the colonic contents. The concentrations of CA, CDCA, αMCA, βMCA, ursodeoxycholic acid and 7-ketodeoxycholic acid were greater and those of TCA and taurodeoxycholic acid were lower in all groups fed the barley-based diets than in the controls ($P<0·001$).

The total amounts of tauroconjugated BA in the intestinal contents are summarised in Table 4. The levels of this BA

Table 3. Steroid groups and total steroids (μmol/g dry matter) in intestinal contents of rats fed the control diet or the barley-based diets A–D for 6 weeks*
(Mean values for ten rats per group and pooled standard errors of the means)

Intestinal contents	Steroid group	Control	Group A	Group B	Group C	Group D	Pooled SEM
Small intestine†	Bile acids	6·45[d]	7·21[c]	9·04[a]	7·76[b]	9·12[a]	0·16
	Neutral sterols	10·67[c]	10·53[c]	12·36[a]	11·42[b]	12·44[a]	0·14
	Total steroids	17·12[c]	17·74[c]	21·40[a]	19·18[b]	21·56[a]	0·28
Caecum	Bile acids	8·14[d]	9·41[c]	11·14[b]	9·66[c]	12·07[a]	0·20
	Neutral sterols	15·46[c]	15·89[c]	19·63[a]	17·47[b]	20·34[a]	0·30
	Total steroids	23·60[e]	25·30[d]	30·77[b]	27·13[c]	32·41[a]	0·29
Colon	Bile acids	9·52[c]	10·11[b]	12·48[a]	10·45[b]	13·06[a]	0·21
	Neutral sterols	18·03[e]	20·61[d]	30·45[b]	25·21[c]	31·04[a]	0·75
	Total steroids	27·55[e]	30·72[d]	42·93[b]	35·66[c]	44·09[a]	0·94

[a,b,c,d,e] Mean values within a row with unlike superscript letters were significantly different ($P<0·01$).
* For details of diets and procedures, see Table 1 and pp. 896–897.
† Second part of the small intestine.

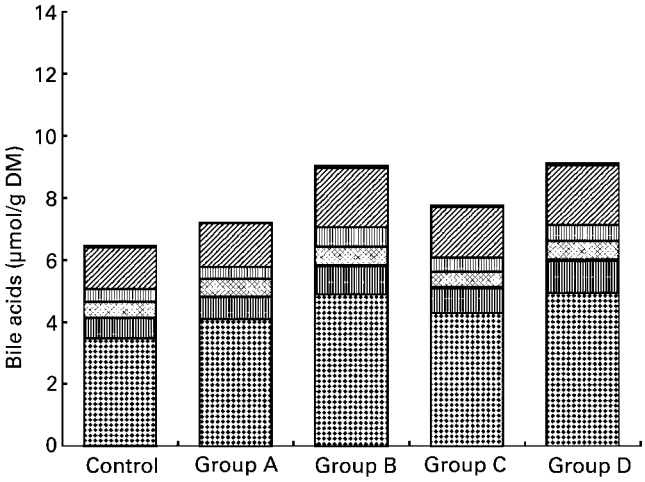

Fig. 1. Bile acids in the contents of the second part of the small intestine of rats fed the control diet or the barley-based diets A–D for 6 weeks. Values are means (*n* 10 per group). (▨), β-Muricholic acid; (▥), α- and ω-muricholic acid; (▩), taurochenodeoxycholic acid; (▥), taurodeoxycholic acid; (▦), taurocholic acid. For details of diets and procedures, see Table 1 and pp. 896–897.

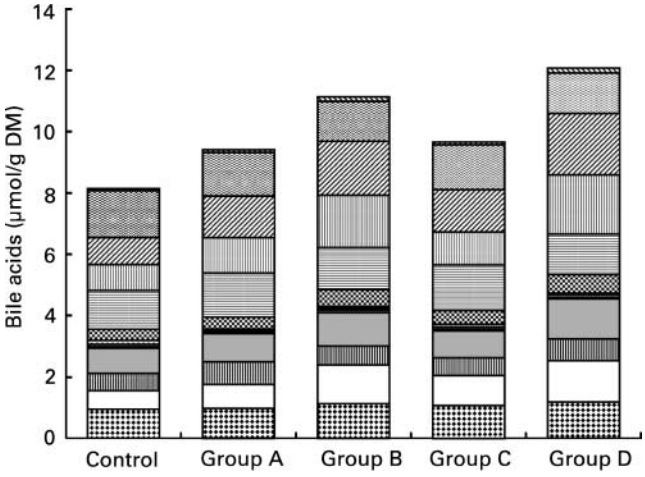

Fig. 2. Bile acids in the caecal contents of rats fed the control diet or the barley-based diets A–D for 6 weeks. Values are means (*n* 10 per group). (▧), Ursodeoxycholic acid; (▩), hyodeoxycholic acid; (▨), β-muricholic acid; (▥), α- and ω-muricholic acid; (▤), lithocholic acid; (■), chenodeoxycholic acid; (▩), taurochenodeoxycholic acid; (⊞), 12-ketolithocholic acid; (▨), 7-ketodeoxycholic acid; (▩), deoxycholic acid; (▤), taurodeoxycholic acid; (□), cholic acid; (▦), taurocholic acid. For details of diets and procedures, see Table 1 and pp. 896–897.

group decreased from the small intestine to colon as a result of enzymic deconjugation by the microflora. Compared with the control group, concentrations of tauroconjugated BA were greater in the contents of the small intestine and caecum ($P<0{\cdot}01$) but lower in the colonic contents ($P<0{\cdot}001$) of all groups fed the barley-based diets.

Amounts of total BA belonging to the CA group (i.e. CA, DCA, 7-ketodeoxycholic acid, 12-ketolithocholic acid) were higher in all groups fed the barley-based diets than in the controls ($P<0{\cdot}001$); highest concentrations were found in groups B and D (Fig. 4). However, the proportion of this BA group was relatively constant between the

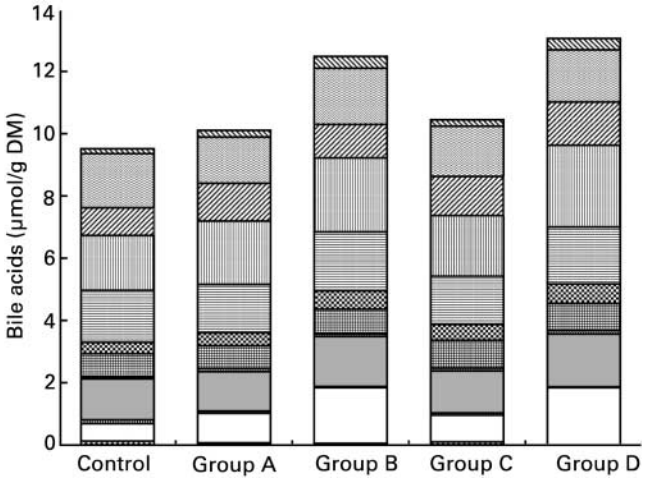

Fig. 3. Bile acids in the colonic contents of rats fed the control diet or the barley-based diets A–D for 6 weeks. Values are means (*n* 10 per group). (▧), Ursodeoxycholic acid; (▩), hyodeoxycholic acid; (▨), β-muricholic acid; (▥), α- and ω-muricholic acid; (▤), lithocholic acid; (■), chenodeoxycholic acid; (▩), taurochenodeoxycholic acid; (⊞), 12-ketolithocholic acid; (▨), 7-ketodeoxycholic acid; (▩), deoxycholic acid; (▤), taurodeoxycholic acid; (□), cholic acid; (▦), taurocholic acid. For details of diets and procedures, see Table 1 and pp. 896–897.

different diets that were fed: 64·0–66·1% in the contents of the second part of the small intestine; 37·2–38·8% in the caecal contents; 30·8–34·9% in the colonic contents. This shows that, independent of the diet that was fed, the structural features of BA, and therefore their physicochemical properties, are important factors for the degree of their reabsorption in the lower parts of the intestinal tract.

The concentrations of total primary BA were greater in all contents of the small intestines, caeca and colons in the groups fed the barley-based diets compared with the controls after 6 weeks ($P<0{\cdot}001$) (Fig. 5). The greatest amounts of primary BA appeared in groups B and D. The concentrations of primary BA decreased from the small intestine to the caecum ($P<0{\cdot}001$).

The concentrations of total secondary BA were greater in all contents of the small intestines in the rats fed the barley-based diets ($P<0{\cdot}001$). In the colonic contents, less secondary BA were found in the colonic contents of group A ($P<0{\cdot}001$) and more secondary BA were present in groups B and D compared with the controls ($P<0{\cdot}002$) (data not shown). The effects of the diets on the proportion of formed secondary BA are shown in Table 5. Only small differences were found between the groups in the contents of the second part of the small intestine (about 10·6%). In contrast, proportions of secondary BA were lower in all caecal and colonic contents of the groups fed the barley-based diets ($P<0{\cdot}001$).

Neutral sterols in intestinal contents

Changes in the composition of NS were observed during their passage through the intestinal tract. In the contents of the second part of the small intestine, higher concentrations of total NS were found in groups B–D compared

Table 4. Tauroconjugated bile acids (μmol/g dry matter) in intestinal contents of rats fed the control diet or the barley-based diets A–D for 6 weeks*

(Mean values for ten rats per group and pooled standard errors of the means)

Intestinal contents	Control	Group A	Group B	Group C	Group D	Pooled SEM
Small intestine†	$4{\cdot}65^{c}$	$5{\cdot}38^{b}$	$6{\cdot}39^{a}$	$5{\cdot}58^{b}$	$6{\cdot}58^{a}$	0·11
Caecum	$1{\cdot}65^{c}$	$1{\cdot}77^{b}$	$1{\cdot}81^{b}$	$1{\cdot}75^{b}$	$1{\cdot}94^{a}$	0·02
Colon	$0{\cdot}24^{a}$	$0{\cdot}14^{b}$	$0{\cdot}09^{c}$	$0{\cdot}17^{b}$	$0{\cdot}02^{d}$	0·01

^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different ($P<0{\cdot}01$).
* For details of diets and procedures, see Table 1 and pp. 896–897.
† Second part of the small intestine.

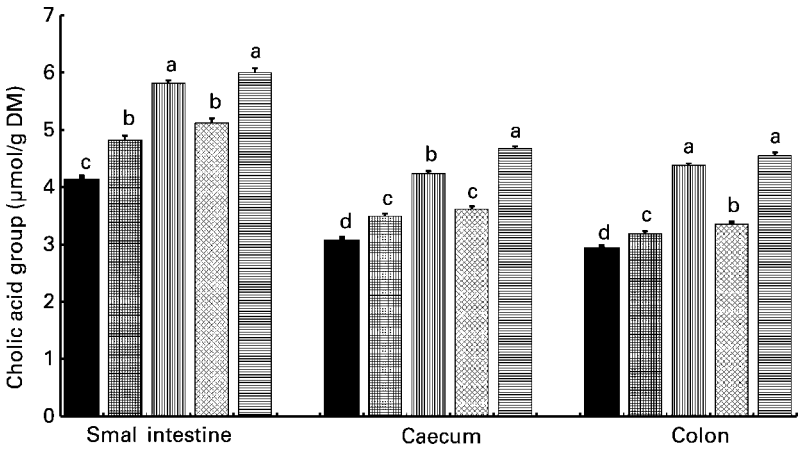

Fig. 4. Bile acids of the cholic acid group in the contents of the second part of the small intestine, caecum and colon of rats fed the control diet (■) or the barley-based diets A (⊞), B (▥), C (▩) and D (▤) for 6 weeks. Values are means, with standard errors of the means represented by vertical bars (*n* 10 per group). ^{a,b,c,d} Mean values with unlike superscript letters were significantly different ($P<0{\cdot}01$). For details of diets and procedures, see Table 1 and pp. 896–897.

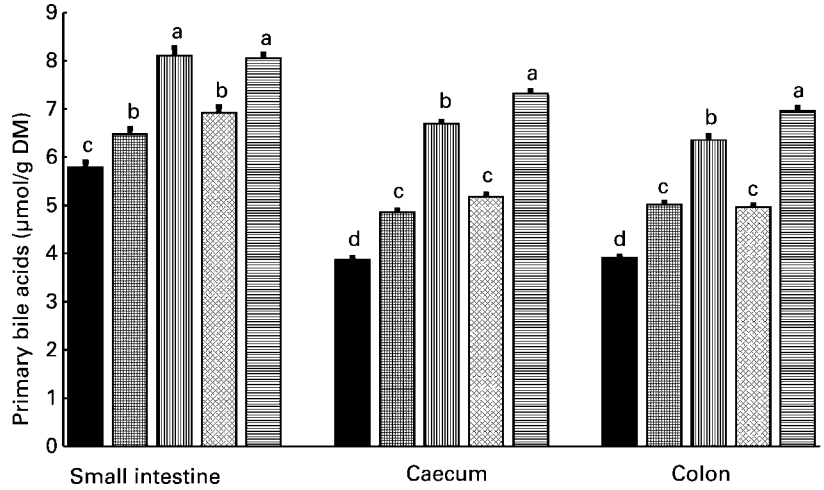

Fig. 5. Primary bile acids in the contents of the second part of the small intestine, caecum and colon of rats fed the control diet (■) or the barley-based diets A (⊞), B (▥), C (▩) and D (▤) for 6 weeks. Values are means, with standard errors of the means represented by vertical bars (*n* 10 per group). ^{a,b,c,d} Mean values with unlike superscript letters were significantly different ($P<0{\cdot}01$). For details of diets and procedures, see Table 1 and pp. 896–897.

with the controls ($P<0{\cdot}005$) (Table 3). Only cholesterol was present here as a NS in distinct amounts (Fig. 6).

Feeding rats the barley-based diets B–D resulted in increased amounts of total NS in the caecal contents compared with the control diet ($P<0{\cdot}001$) (Table 3). As a result of microbial conversions, coprostanol appeared, besides cholesterol, in higher concentrations in groups B–D ($P\leq0{\cdot}007$) compared with the control group. Coprostanone was present only in negligible amounts (Fig. 6).

In the colonic contents of the rats fed the barley-based diets, higher concentrations of total NS were found ($P<0{\cdot}001$); feeding of diets B and D was most effective

Table 5. Proportions of secondary bile acids (%) in intestinal contents of rats fed the control diet or the barley-based diets A–D for 6 weeks*

(Mean values for ten rats per group and pooled standard errors of the means)

Intestinal contents	Control	Group A	Group B	Group C	Group D	Pooled SEM
Small intestine†	10·24^{c}	10·20^{c}	10·31^{c}	10·75^{b}	11·68^{a}	0·10
Caecum	52·40^{a}	48·43^{b}	39·92^{d}	46·41^{c}	39·35^{e}	0·72
Colon	58·85^{a}	50·37^{b}	49·10^{c}	52·45^{a}	46·68^{d}	0·59

^{a,b,c,d,e} Mean values within a row with unlike superscript letters were significantly different ($P < 0·01$).
* For details of diets and procedures, see Table 1 and pp. 896–897.
† Second part of the small intestine.

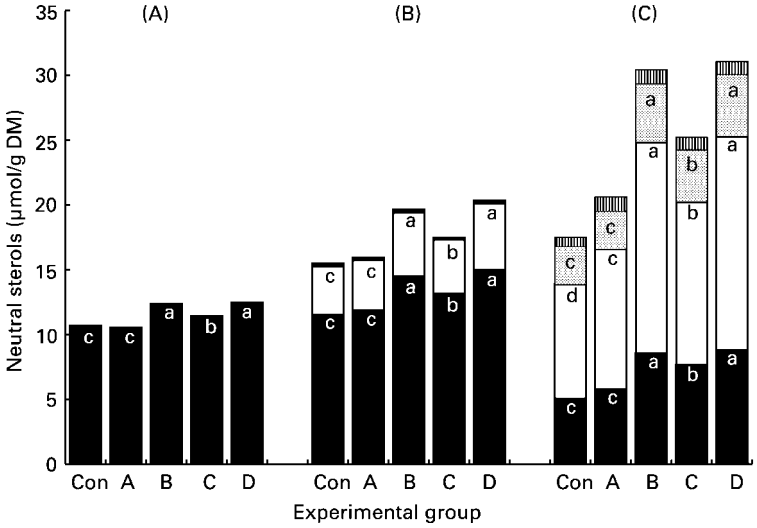

Fig. 6. Effects of the control diet (Con) or the barley-based diets A–D on the concentrations of cholesterol (■), coprostanol (□), coprostanone (▨) and cholestanone (▥) in the contents of the second part of the small intestine (A), caecum (B) and colon (C) of rats after a feeding period of 6 weeks. Values are means (n 10 per group). ^{a,b,c,d}Mean values with unlike superscript letters were significantly different ($P<0·01$). For details of diets and procedures, see Table 1 and pp. 896–897.

(Table 3). The spectrum of NS was broader in the colonic contents than in other parts of the intestinal tract. Whereas the amount of cholesterol decreased in the colon compared with the caecum, higher concentrations of coprostanol, coprostanone and cholestanone were found in the colonic contents. Coprostanol was the dominant NS here (50–55%). The proportions of the individual NS were relatively constant in all rat groups. Feeding the barley-based diets B–D resulted in higher concentrations of all individual NS compared with the controls ($P<0·001$; cholestanone, $P\leq 0·04$) (Fig. 6).

The amount of NS in the lower parts of the intestinal tract was positively affected especially by the presence of macromolecular viscous DF such as β-glucan. The microbial conversion of cholesterol to its metabolites seems to be enhanced indirectly as a result of DF fermentation.

Total steroids in intestinal contents

Finally, concentrations of total steroids were evaluated in the intestinal contents. NS dominated in the intestinal contents as steroids (Table 3). Altogether, BA amounted to approximately 30–40% of the total steroids found. Up to 22 and 32 μmol total steroids/g DM appeared in the sixth week of the experiment in the small-intestinal and caecal contents of the groups fed the barley-based diets, respectively. In the colonic contents, the amounts of total steroids increased from about 27 μmol/g DM (control) to about 44 μmol/g DM (group D). Compared with the control group, concentrations of total steroids were higher in the contents of the second part of the small intestines in groups B–D as well as in the caecal and colonic contents of all groups fed the barley-based diets ($P<0·001$).

Discussion

The barley products used in diets A–D were more or less related to the wholegrain type. Therefore, the physiological effects of isolated or purified DF components were not tested in the present study. Besides the cell-wall polysaccharides of the barley meals, RS was generated by an optimised extrusion process (Huth *et al.* 2000). Recently, Vasanthan *et al.* (2002) also discussed the formation of RS during the extrusion of barley meal. In addition to the extrudates, the present study used a combination of meal from the β-glucan-rich barley variety 'Prowashonupana' and a commercial RS preparation in the feeding experiments. It was reported that the extrusion of barley resulted in an increased extract viscosity and water-binding

capacity (Vranjes & Wenk, 1995). Such functional properties of the DF-rich preparations play a role in the physiological effects observed during their passage through the gastrointestinal tract (Lund *et al.* 1989; Doublier & Wood, 1995; Tejinder *et al.* 2000). Thus, carbohydrate digestion from a β-glucan-enriched barley ('Prowashonupana') was reduced in human subjects (Lifschitz *et al.* 2002).

The barley-based diets that were fed were shown to have several beneficial effects in rats. Thus, the amounts of excreted BA and NS were higher in the groups fed the barley-containing diets during the present experiment compared with a diet containing no barley. Consequently, the normal intestinal absorption of BA (Hofmann, 1994) was partly disturbed by DF. Further, the proportion of secondary BA was lower in the faeces when greater amounts of highly fermentable DF were present in the diets. The dominating NS component excreted was coprostanol (Dongowski *et al.* 2002).

The analysed steroid concentrations in the intestinal contents and faeces are given on a dry-weight basis. The concentrations calculated on the basis of the overall amounts in digesta resulted practically in the same order (data not shown).

The molecular weight of the β-glucans seems to play a role in their effects on steroid excretion and on the decrease of plasma lipids. But the molecular weight (and the viscosity) may be affected by technological treatments during the preparation of the diets and the physico-chemical conditions in the stomach and small intestine (Sundberg *et al.* 1996). The optimised conditions used during extrusion, the inclusion of the β-glucans in the wholegrain matrices as well as the absence of β-glucanase activities in the barley samples suppressed the degradation of the β-glucans in applied extrudates (Huth *et al.* 2000). However, the molecular weight of β-glucan may partly be reduced during its passage through the upper gastrointestinal tract (Johansen *et al.* 1993, 1997; Robertson *et al.* 1997). Bach Knudsen *et al.* (1993) found no quantitative losses of β-glucan from oat flour or bran in the stomach and in the first, middle and distal thirds of the small intestine in ileum-cannulated pigs, while in the terminal ileum digestibility increased. Lia *et al.* (1995) reported that the 24 h excretion of BA and cholesterol was greater in ileostomy subjects given macromolecular β-glucan-containing diets. Interactions between BA and viscous DF are evidently the reason for this effect. The same effect was found in the authors' experiments. The nature of the interactions between BA and β-glucan in the small intestine is not known. Direct linkages are unlikely (Bowles *et al.* 1996). In the case of pectin, it was found *in vitro* that the interactions are preferentially of a hydrophobic nature and that they are affected by the structure of both the DF and the BA as well as by the physicochemical conditions (Dongowski, 1995, 1997). A further aspect is interactions between the steroids and active sites of the more or less intact cell wall of the plant materials (Dongowski & Ehwald, 1999).

Feeding the barley-based diets resulted in higher levels of BA in the intestinal contents, especially when β-glucan-rich diets were given. Reasons for this effect may be, besides the discussed direct interaction between the steroids and DF, the higher viscosity in the gut, a possible disturbance of micelle formation and lipid digestion and/or the hindrance of BA reabsorption in the presence of DF. The permanent alterations in the composition of the BA during their passage through the intestinal tract are a result of several enzymic actions by the microflora on the original BA. The first step is the deconjugation of the (tauro)conjugated BA (Baron & Hylemon, 2000) under the formation of 'free' (not conjugated) BA. Then the OH group at C-atom 7 of the steroid nucleus is removed by the action of 7α-dehydroxylase; i.e. this step is the partial formation of secondary BA from the primary BA which are formed in the hepatocytes (Hofmann, 1999). Intestinal bacteria with 7α-dehydroxylating activities belonging to the genera *Eubacterium* and *Clostridium* are also known (Baron & Hylemon, 2000). Other enzymic actions lead to the insertion of OH groups at C-atom 6, the changing of OH groups from the α- into the β-position or the formation of keto groups from OH groups (Baron & Hylemon, 2000).

It is not yet clear whether steroids are involved in large-bowel carcinogenesis (Roy *et al.* 1999) and reports in the literature are conflicting (Hofstad *et al.* 1998). Especially, certain secondary BA are considered to be cytotoxic and to be promoting factors in colon carcinogenesis (Owen, 1997). BA may be cytotoxic when present in abnormally high concentrations. This cytoxicity is strongly affected by the structure of the BA. The greater the hydrophobicity, the greater the cytotoxicity (Hofmann, 1999). Thus, LCA and DCA but also CDCA show a higher hydrophobicity than CA. The detergent activity of BA may diminish the protection of the mucin layer and damage the membranes of surface epithelial cells in the colon. BA in faecal water are more important for their toxicity to epithelial cells than the total BA concentration (Roberton, 1993). Especially LCA and DCA have been shown to be carcinogenic by the Ames test or other *in vitro* experiments or in animal models (Salter *et al.* 1996). Higher BA concentrations in the lumen can activate cellular protein kinase C which stimulates cell proliferation. Compared with a combined DCA–butyrate treatment, the incubation of biopsies from human colon tissue with DCA alone resulted in a significantly higher crypt-labelling index (Bartram *et al.* 1994). Butyrate is a product of the bacterial fermentation of DF. It was shown in a former study (Dongowski *et al.* 2002) that concentrations of butyrate in the intestinal contents of rats were significantly higher ($P<0{\cdot}05$) when barley-based diets were fed. McMillan *et al.* (2000) found that DCA and CDCA, at levels present in the colon, gave a modest increase in cell proliferation and decreased spontaneous apoptosis in adenoma cells. BA inhibited the induction of apoptosis by butyrate but this effect could be overcome by increasing the butyrate concentration.

In the presence of DF, the problematical effects of BA in the lower parts of the intestinal tract may be suppressed in different ways, i.e. by lowering the proportion of secondary BA, by decreasing the transit time of the gut, by the dilution or inclusion of the BA and by the improvement of the health of the colonic mucosa. DF-rich diets

are obviously the best strategy to influence positively the excretion of BA and to stabilise both a healthy microflora and a healthy mucosa. The decrease in intestinal pH by up to one unit (Dongowski *et al.* 2002) is an effect of the fermentation of both conventional DF and RS and the formation of short-chain fatty acids (Fadden *et al.* 1997). A lower pH in the caecal and colonic contents leads to a partial inhibition of the bacterial 7α-dehydroxylase activity resulting in lower proportions of secondary BA in the experimental groups compared with the control group.

Another aspect is the higher concentration of NS in the intestinal contents of the rats fed the barley-containing diets. In the same way as the BA, greater amounts of these steroids were transported into the caecum and colon and also excreted in higher quantities especially when more macromolecular viscous DF, such as β-glucan, were present in the gut (Dongowski *et al.* 2002). The partial microbial transformation of cholesterol resulted in the formation of coprostanol, and then in the formation of cholestanone and coprostanone as major metabolites. It is not yet clear whether NS may also be involved in colon carcinogenesis. In a previous study, increased faecal concentrations of cholesterol were found in patients with large adenomas and colon cancer (Roy *et al.* 1999). In azoxymethane-treated rats, diets supplemented with 1% cholesterol and 0·15% DCA resulted in higher faecal steroid concentrations and in elevated colonic cell proliferation (Hori *et al.* 1998).

In contrast to the control group, the concentration of total faecal BA and NS increased continuously during the feeding period from week 0 to week 6 in all groups fed the barley-containing diets. In groups B and D, this increase was significantly different from the control group even after 2 weeks ($P < 0{\cdot}05$) (Dongowski *et al.* 2002).

In addition to the discussed effects on the formation of secondary BA, the fermentation of DF by the intestinal microflora may affect the composition of steroids in the lower part of the intestinal tract. Greater numbers of such bacteria were found in the caecum and colon, which are able to split and use DF, when greater amounts of fermentable DF are present in the lower parts of the intestinal tract.

Besides the viscous and highly fermentable DF components, some other constituents of the wholegrain barley products may affect steroid metabolism: phytosterols, tocotrienols and/or α-linolenic aid as shown in several studies (Lupton *et al.* 1994; McIntosh *et al.* 1995; Jadhav *et al.* 1998; Kerckhoffs *et al.* 2002; Moreau *et al.* 2002; Trautwein *et al.* 2002). But concentration of phytosterols is relatively low in barley compared with maize products or maize oil. Therefore the present study looked especially at the effects of selected soluble DF components.

In conclusion, barley-based products rich in β-glucan and RS have beneficial effects on the steroid pattern in rats. Because of their structural and functional properties (saccharide composition, water binding, viscosity, etc.), they are able to interact with steroids and to transport greater amounts of BA and NS towards lower parts of the intestinal tract. The composition of the individual steroids in the caecal and colonic contents is also affected by the fermentation of the DF components present in the gut. In the lower parts of the intestinal tract, the bulking ability of the unfermentable or slowly fermentable DF fraction and the higher bacterial mass affect the amount of excreted steroids. The greater excretion of both steroid groups, BA and NS, in the presence of the higher contents of barley DF may be important for the reduction of lipids in the serum of hypercholesterolaemic individuals. Despite higher BA concentrations in the colon in the presence of DF, their contact with the mucosa is certainly diminished (reduced transit time, inclusion in the gut, etc.). The colonic mucosa is less damaged if DF are consumed regularly and in sufficient amounts. Further, the higher consumption of both highly fermentable and slowly fermentable DF may counteract the involvement of certain BA in the malignant tumour formation of colonic epithelial cells. However, the involved complex mechanisms are not yet fully understood.

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