

## Effects of oat and rye fractions on biliary and faecal bile acid profiles in Syrian golden hamsters (*Mesocricetus auratus*)

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The effects of bran and starchy endosperm fractions of oat and rye on faecal weight and on biliary and faecal bile acids were studied in Syrian golden hamsters (*Mesocricetus auratus*). The animals fed on diets supplemented with steam-flaked oat bran, oat bran or rye bran had higher wet and dry weights of faeces compared with the animals fed on the fibre-free or low-fibre endosperm diets. A higher mean percentage of biliary cholic acid and a lower mean percentage of chenodeoxycholic and lithocholic (LCA) acids was observed in the bran-supplemented dietary groups. Animals fed on the bran-supplemented diets had increased daily faecal excretion of both total saponifiable and total free bile acids compared with the animals fed on fibre-free or endosperm-supplemented diets. The mean percentage of total saponifiable bile acids in the faeces was higher, and that of free bile acids lower in the animals fed on bran-supplemented diets. A significantly lower concentration of faecal free LCA was observed in the animals fed on the rye-bran diet. Both bran and endosperm diets reduced the faecal LCA:deoxycholic acid compared with the fibre-free diet, but the bran diets had a more pronounced effect than endosperm diets.

Oats: Rye: Bile acids: Hamsters

Oat bran is a well-known cereal source of dietary fibre which is rich in partly water-soluble, mixed-linked  $\beta$ -glucan (Henry, 1987; Shinnick *et al.* 1988) and possesses hypocholesterolaemic properties both in experimental animals and in humans (Chen & Anderson, 1981; Gold & Davidson, 1988; Anderson *et al.* 1990; Qureshi *et al.* 1991). The hypocholesterolaemic effect of oats may be related to an increased faecal excretion of bile acids (Judd & Truswell, 1981; Ilman & Topping, 1985). Since bile acids and their derivatives from bacterial action have been suggested to be a source of carcinogens responsible for colo-rectal cancer (Owen *et al.* 1987; Nair, 1988), the excessive bile acid excretion induced by oats has aroused the suspicion that oats might play a role in carcinogenesis of the colon (Hurt *et al.* 1988). For this reason it is of interest to investigate how oats affect bile acid metabolism and the bile acid profile in the faeces.

In most early studies only primary and secondary bile acids have been measured. However, recently it was shown that up to 80% of the bile acids may occur in the form of a saponifiable fraction (esterified bile acids) and that the degree of esterification depends on the diet (Korpela *et al.* 1988, 1992). Therefore, we used this new methodology (Korpela *et al.* 1986) for the present study.

Untreated oat products may deteriorate during long storage. To keep the products in good condition for a longer time, heat treatment by steam-flaking is commonly performed. Whether the heat treatment interferes with the physiological effects of oats is not clear. Rye has a different dietary fibre composition compared with that of oats, and less is known about its physiological effect.

The aim of the present study was to investigate the effect of oat and rye fractions on the composition of bile acids in bile, on the profile of bile acids in faeces, and to elucidate the possible role that oat and rye fractions may play in bile acid metabolism in the gut.

## MATERIALS AND METHODS

### *Diets and nutrient analysis*

The experimental control diet was a fibre-free diet (FF) prepared from extruded wheat starch, low-fat milk powder (S67; Semper AB, Stockholm, Sweden) and olive oil. The experimental low-fibre diets (LFD) were prepared with the same basic components as those in the FF diet plus starchy endosperm fractions: either heat-treated oat outer endosperm (HOE1), heat-treated oat inner endosperm (HOE2), oat inner endosperm (OE2) or rye endosperm (RE). The experimental high-fibre diets (HFD) were also made with the same basic components as those in the FF diet plus heat-treated oat bran (HOB), oat bran (OB) or rye bran (RB). The fibre content in the LFD was adjusted to 20 g/kg and that in the HFD to 120 g/kg. The procedures for processing and separating oat fractions, oat bran, outer and inner endosperms have been described in detail by Westerlund *et al.* (1992). The nutrient contents of the oat and rye fractions are listed in Table 1. The dietary fibre contents of the fractions were analysed by the method described by Theander & Westerlund (1986) and the mixed-linked  $\beta$ -glucan content by the method of Åman & Graham (1987). The crude protein ( $N \times 6.25$ ) was analysed by the standard method (Association of Official Analytical Chemists, 1990) and crude fat by the official method of the European Communities (Anon, 1984). All diets were isoenergetic in terms of percentage energy content from the nutrients: 20% of the energy from protein, 30% from fat and 50% from carbohydrates (excluding the energy values contained in dietary fibre and derived from fermentation). Oat fractions were provided by Graminae AB, Lidköping and rye fractions by Wasabröd AB, Filipstad, Sweden. The composition and the nutritional content of the diets are listed in Tables 2 and 3.

### *Experimental procedures*

Male Syrian golden hamsters (*Mesocricetus auratus*), 4–6 weeks old (Bamting & Kingman, Hull, England), were randomized into eight groups, each containing thirteen animals. The animals were individually placed in plastic cages with perforated middle layer and had free access to water and the experimental diets.

After an adaptation for 1 week with conventional laboratory diet (R3-EWOS-ALAB Brood Stock Feed; EWOS AB, Södertälje, Sweden) the hamsters were fed on the experimental diets for 6 weeks. After 4 weeks of feeding the experimental diets the faeces of the individual animals were collected every 24 h on three consecutive days. Each 24 h faeces collection was weighed, frozen at  $-70^\circ$  and freeze-dried before chemical analysis. At the end of the experimental period the animals were anaesthetized with intraperitoneal sodium pentobarbital (0.03 mg/g body weight) and a midline laparotomy was performed. After ligation of the common bile duct for 25 min the gall bladder was removed and the bile in the gall bladder collected. The collected bile samples were frozen at  $-70^\circ$  and kept frozen until analysed.

Table 1. *Nutrient content of the fractions of oat and rye (g/kg)*

Cereal fraction	Protein	Fat	Carbohydrates*	Dietary fibre			Ash
				Total	$\beta$ -glucan	Moisture	
HOB	137	107	396	204	74	122	34
HOE1	87	64	661	52	19	127	10
HOE2	80	60	681	41	20	129	9
OB	143	102	416	197	70	112	34
OE2	81	62	689	40	18	119	9
RB	123	36	476	198	24	142	25
RE	45	10	752	45	7	144	4

HOB, heat-treated oat bran; HOE1, HOE2, heat-treated oat outer and inner endosperm respectively; OB, oat bran; OE2, oat inner endosperm; RB, rye bran; RE, rye endosperm.

\* Calculated value for carbohydrates excluding dietary fibre.

Table 2. *The composition of the experimental diets (g/kg)*

Diet	S67*	Olive oil	Starch	Vitamin mixture	Mineral mixture	Gelatin	Cereal fraction added
HOB	113	49	180	10	50	10	588
HOE1	221	104	201	12	60	10	392
HOE2	215	99	116	12	60	10	488
OB	102	50	156	10	50	10	622
OE2	212	98	108	12	60	10	500
RB	120	90	114	10	50	10	606
RE	241	123	110	12	60	10	444
FF	281	132	505	12	60	10	0

HOB, heat-treated oat bran; HOE1, HOE2, heat-treated oat outer and inner endosperm respectively; OB, oat bran; OE2, oat inner endosperm; RB, rye bran; RE, rye endosperm; FF, fibre-free.

\* Low-fat milk powder; Semper AB, Sweden.

Table 3. *Nutrient and energy contents of the experimental diets*

Diet	Nutritional content (g/kg)			Dietary fibre (g/kg)		Energy (%) from		
	Protein	Fat	Carbohydrate*	Total	$\beta$ -glucan	Protein	Fat	Carbohydrate*
HOB	165	114	411	120	435	20.0	30.0	50.0
HOE1	191	132	477	20	74	20.0	30.1	49.9
HOE2	192	131	475	20	98	20.1	30.0	49.9
OB	166	115	414	120	435	20.0	30.1	49.9
OE2	191	132	479	20	90	20.0	29.9	50.1
RB	164	114	411	120	145	19.9	30.0	50.1
RE	190	131	476	20	31	20.0	30.0	50.0
FF	197	136	494	0	0	20.0	30.0	50.0

HOB, heat-treated oat bran; HOE1, HOE2, heat-treated oat outer and inner endosperm respectively; OB, oat bran; OE2, oat inner endosperm; RB, rye bran; RE, rye endosperm; FF, fibre-free.

\* Calculated value for carbohydrates excluding dietary fibre.

*Chemical analyses of biliary and faecal bile acids*

The total content and composition of bile acids were measured by GLC after alkaline treatment of bile samples with subsequent extraction of free acids according to the method of Bosaeus *et al.* (1986), followed by preparation of corresponding methyl esters by the method of Shaw & Elliott (1978). The GLC analyses were performed on a Hewlett Packard 5890 instrument fitted with an auto-injector and an SE-52 column (25 m × 0.25 mm i.d., helium flow 1.5 ml/min) which was first held at 250° for 6 min and then heated to 280° at a rate of 1.0°/min. The GLC was calibrated with authentic samples of the bile acids and the internal standards stigmaterol and 5- $\beta$ -cholanic acid. Calculation of raw data was performed using Model 2600 chromatography software from Nelson Analytical Inc. (Cupertino, CA, USA). The multicomponent analysis of the faecal bile acids was accomplished by the GLC method of Korpela *et al.* (1988).

*Statistics*

Data were expressed as means and standard deviations and subjected to analysis of variance before the evaluations were performed with Student's *t* test between different groups. Pearson's coefficient of correlation was used for selected variables.

## RESULTS

*Daily excretion of faeces*

Wet and dry weights of faeces excreted daily for the dietary groups supplemented with bran (HOB, OB and RB) were higher than those for the FF dietary group (fibre-free;  $P = 0.000$ ) and the corresponding groups supplemented with oat or rye endosperm (HOE1, HOE2, OE2 and RE;  $P = 0.000$ ; Table 4). A higher faecal moisture content was observed in the RB dietary group than in group FF ( $P = 0.001$ ).

*Bile acids in the bile*

No significant difference in the concentration of biliary total bile acids was observed between the dietary groups (Table 5). For groups HOB and OB the concentrations of chenodeoxycholic (CDCA), deoxycholic (DCA) and lithocholic (LCA) acids were lower than those for the FF dietary group (HOB group  $P = 0.006$ ,  $P = 0.033$  and  $P = 0.004$ , respectively; OB group  $P = 0.007$ ,  $P = 0.022$  and  $P = 0.003$ , respectively), and for the corresponding endosperm-supplemented diets HOE1 (HOB,  $P = 0.014$ ,  $P = 0.032$  and  $P = 0.003$  respectively), HOE2 (HOB group,  $P = 0.001$ ,  $P = 0.002$  and  $P = 0.000$ , respectively) and OE2 (OB group,  $P = 0.014$ ,  $P = 0.002$  and  $P = 0.002$ , respectively). In the RB dietary group the CDCA concentration was lower than that for the FF group ( $P = 0.015$ ), and LCA was lower than that for the FF ( $P = 0.005$ ) and RE ( $P = 0.005$ ) groups.

The proportion of biliary cholic acid (CA) relative to total bile acids for the bran groups (HOB, OB and RB) was higher than those for the FF ( $P = 0.000$  respectively) and corresponding endosperm (HOE1, HOE2, OE2 and RE) dietary groups ( $P = 0.000$  respectively) but the proportion of CDCA was lower than that for the FF ( $P = 0.000$ ,  $P = 0.001$  and  $P = 0.001$ ) and corresponding endosperm HOE1 ( $P = 0.001$ ), HOE2 ( $P = 0.000$ ), OE2 ( $P = 0.023$ ) and RE ( $P = 0.000$ ) dietary groups (Table 6). The proportion of biliary LCA relative to total bile acids for the bran groups (OB and RB) was lower than that for the FF dietary group ( $P = 0.001$  and  $P = 0.002$ , respectively) and those for the corresponding endosperm OE2 ( $P = 0.021$ ) and RE ( $P = 0.023$ ) groups, but for the heat-treated oat bran group (HOB) it was only lower than that for the FF dietary group ( $P = 0.017$ ).

Table 4. *Effect of oat and rye fractions on wet and dry weight of the faeces in Syrian golden hamsters (Mesocricetus auratus)*

(Mean values and standard deviations for thirteen hamsters/group)

Dietary group‡	Faecal wt (g/d)					
	Wet wt		Dry wt		Moisture	
	Mean	SD	Mean	SD	Mean	SD
HOB	0.64*	0.10	0.61*	0.10	0.03	0.01
HOE1	0.46†	0.12	0.43†	0.11	0.03	0.01
HOE2	0.41†	0.07	0.38†	0.07	0.02	0.01
OB	0.77*	0.09	0.74*	0.09	0.03	0.01
OE2	0.48†	0.11	0.45†	0.11	0.03	0.02
RB	0.89*	0.12	0.84*	0.13	0.05*	0.03
RE	0.45†	0.07	0.42†	0.07	0.02†	0.01
FF	0.39	0.11	0.36	0.11	0.03	0.01

HOB, heat-treated oat bran; HOE1, HOE2, heat-treated oat outer and inner endosperm respectively; OB, oat bran; OE2, oat inner endosperm; RB, rye bran; RE, rye endosperm; FF, fibre-free.

Mean values were significantly different from those for the FF dietary group: \*  $P < 0.05$ .

Mean values were significantly different from those for the corresponding bran dietary group: †  $P < 0.05$ .

‡ For details of composition, see Tables 1–3.

Table 5. *Effect of oat and rye fractions on the concentration of biliary bile acids in Syrian golden hamsters (Mesocricetus auratus)*

(Mean values and standard deviations for thirteen hamsters/group)

Dietary group‡	Concentration of biliary bile acids (mmol/l)									
	Cholic		Chenodeoxycholic		Deoxycholic		Lithocholic		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HOB	20.2	7.6	9.0*	4.3	4.7*	2.1	0.6*	0.2	34.4	13.3
HOE1	19.1	3.3	13.1†	3.5	6.5†	2.0	0.9†	0.3	39.5	6.3
HOE2	23.0	7.1	15.7†	4.1	7.4†	2.0	1.0†	0.3	47.1	11.6
OB	22.1	4.9	9.7*	3.0	4.8*	1.4	0.6*	0.2	37.1	7.6
OE2	23.2	5.4	15.0†	6.5	7.5†	2.5	0.9†	0.4	46.7	12.1
RB	22.5	4.8	10.3*	2.5	5.9	1.9	0.6*	0.1	39.2	7.0
RE	18.7	6.4	13.7	4.6	6.1	2.5	1.0†	0.6	39.4	12.7
FF	19.0	5.8	14.8	6.0	6.7	2.6	1.1	0.6	42.6	13.6

HOB, heat-treated oat bran; HOE1, HOE2, heat-treated oat outer and inner endosperm respectively; OB, oat bran; OE2, oat inner endosperm; RB, rye bran; RE, rye endosperm; FF, fibre-free.

Mean values were significantly different from those for the FF dietary group: \*  $P < 0.05$ .

Mean values were significantly different from those for the corresponding bran dietary group: †  $P < 0.05$ .

‡ For details of composition, see Tables 1–3.

#### *Bile acids in the faeces*

The animals fed on the bran-supplemented diets (HOB, OB and RB) had higher daily excretions of total saponifiable (SpBA), total free (FBA) and, thus, total analysed bile acids (TBA; SpBA + FBA) in faeces compared with those for the animals fed on the FF ( $P = 0.000$  respectively) and corresponding endosperm-supplemented diets, HOE1

Table 6. Effect of oat and rye fractions on the amounts of biliary individual bile acid relative to total bile acids and biliary lithocholic (LCA):deoxycholic (DCA) acid in Syrian golden hamster (*Mesocricetus auratus*)

(Mean values and standard deviations for thirteen hamsters/group)

Dietary group†	Amounts biliary individual bile acid (% total bile acids)									
	Cholic		Chenodeoxycholic		Deoxycholic		Lithocholic		LCA:DCA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HOB	59.1*	3.7	25.3*	4.8	13.8	3.7	1.8*	0.6	0.14	0.05
HOE1	48.4†	5.3	32.9†	5.6	16.5	4.9	2.2	0.6	0.14	0.04
HOE2	48.2†	4.2	33.3†	3.4	16.2	4.3	2.3	0.7	0.14	0.04
OB	59.6*	5.6	25.9*	5.5	13.0	3.3	1.5*	0.3	0.12*	0.04
OE2	50.1†	5.4	31.2†	5.1	16.6	5.4	2.1†	0.7	0.13	0.03
RB	57.1*	3.1	26.2*	4.5	15.2	5.1	1.5*	0.4	0.11*	0.04
RE	47.4†	4.2	34.6†	4.7	15.4	3.5	2.6†	1.5	0.17†	0.09
FF	46.0	5.0	35.2	7.1	16.3	4.9	2.5	1.0	0.17	0.07

HOB, heat-treated oat bran; HOE1, HOE2, heat-treated oat outer and inner endosperm respectively; OB, oat bran; OE2, oat inner endosperm; RB, rye bran; RE, rye endosperm; FF, fibre-free.

Mean values were significantly different from those for the FF dietary group: \*  $P < 0.05$ .

Mean values were significantly different from those for the corresponding bran dietary group: †  $P < 0.05$ .

‡ For details of composition, see Tables 1-3.

( $P = 0.000$ ,  $P = 0.000$  and  $P = 0.000$ , respectively), HOE2 ( $P = 0.000$ ,  $P = 0.000$  and  $P = 0.000$ , respectively), OE2 ( $P = 0.000$ ,  $P = 0.034$  and  $P = 0.010$ , respectively), and RE ( $P = 0.000$ ,  $P = 0.006$  and  $P = 0.002$ , respectively; Table 7).

In the animals fed on oat-bran-supplemented diets (HOB and OB) the faecal concentrations of total SpBA were higher than those for the animals fed on the FF ( $P = 0.000$ ) and corresponding endosperm-supplemented HOE1 ( $P = 0.000$ ), HOE2 ( $P = 0.000$ ) and OE2 ( $P = 0.003$ ) diets. In the animals fed on the rye-bran diet (RB) it was also higher than that for the animals fed on the FF diet ( $P = 0.009$ ). The concentration of total FBA for the animals fed on heat-treated oat bran (HOB) was higher than that for the animals fed on the FF ( $P = 0.001$ ) and inner endosperm diets (HOE2,  $P = 0.032$ ). No difference was found in the concentration of the total FBA between the animals fed on the heat-untreated bran diets (OB and RB) and those fed on the FF and corresponding OE2 and RE diets. The faecal concentrations of TBA were higher in the animals fed on oat-bran diets (HOB and OB) than for the animals fed on the FF ( $P = 0.000$  and  $P = 0.028$ , respectively) and corresponding endosperm diets, HOE1 ( $P = 0.016$ ) and HOE2 ( $P = 0.006$ ). No difference was found in the concentration of the TBA between the animals fed on the rye-bran diet (RB) and those fed on the FF and RE diets (Table 7).

The proportion of total SpBA relative to TBA was significantly higher for the animals fed on bran-supplemented diets (means and SD, %) HOB 11.9 (SD 5.3), OB 11.6 (SD 4.4) and RB 12.6 (SD 7.4), than that for the animals fed on the FF diet, 6.2 (SD 2.8) ( $P = 0.002$ ,  $P = 0.001$  and  $P = 0.000$  respectively), and the corresponding endosperm-supplemented diets, HOE1 5.0 (SD 2.2) ( $P = 0.000$ ), HOE2 5.9 (SD 1.4) ( $P = 0.001$ ), OE2 5.6 (SD 2.5) ( $P = 0.000$ ) and RE 7.0 (SD 2.6) ( $P = 0.018$ ). The proportion of total FBA relative to TBA was significantly lower for the animals fed on bran diets, HOB 88.1 (SD 5.3), OB 88.4 (SD 4.4) and RB 87.4 (SD 7.4), than that for the animals fed on the FF diet, 93.8 (SD 2.8) ( $P = 0.002$ ,  $P = 0.001$  and  $P = 0.008$ , respectively) and endosperm-supplemented diets, HOE1 95.0

Table 7. Effect of oat and rye fractions on daily excretion and concentration of total saponifiable, total free and total analysed bile acids in faeces of Syrian golden hamsters (*Mesocricetus auratus*)

Mean values and standard deviations for thirteen hamsters/group

Dietary group‡	Daily bile acid excretion (nmol/d)						Bile acid concentration (nmol/g)					
	Saponifiable		Free		Analysed		Saponifiable		Free		Analysed	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HOB	253*	124	1878*	494	2131*	557	405*	174	3085*	645	3490*	670
HOE1	56†	27	1093†	309	1149†	319	136†	63	2629	731	2765†	755
HOE2	61†	32	954†	339	1015†	367	156†	63	2481†	704	2637†	755
OB	249*	99	1936*	61	2185*	666	338*	131	2637	749	2975*	812
OE2	84†	42	1438*†	517	1522*†	540	190†	98	3244*	999	3434	104
RB	203*	87	1665*	771	1868*	817	247*	108	1925	729	2172	762
RE	70†	36	952†	361	1022†	379	168	96	2242	775	2410	826
FF	54	39	820	428	874	456	142	77	2143	649	2285	686

HOB, heat-treated oat bran; HOE1, HOE2, heat-treated oat outer and inner endosperm respectively; OB, oat bran; OE2, oat inner endosperm; RB, rye bran; RE, rye endosperm; FF, fibre-free.

Mean values were significantly different from those for the FF dietary group: \*  $P < 0.05$ .

Mean values were significantly different from those for the corresponding bran dietary group: †  $P < 0.05$ .

‡ For details of composition, see Tables 1–3.

(SD 2.2) ( $P = 0.000$ ), HOE2 94.1 (SD 1.4) ( $P = 0.001$ ), OE2 94.4 (SD 2.5) ( $P = 0.000$ ) and RE 93.0 (SD 2.6) ( $P = 0.018$ ).

When considering the sub-fractions of the individual bile acids, increased concentrations of saponifiable LCA, epi-deoxycholic acid (EDCA) and DCA were observed in the animals fed on the oat-bran diets (HOB and OB) compared with the FF diet (HOB group  $P = 0.002$ ,  $P = 0.000$  and  $P = 0.000$ , respectively; OB group  $P = 0.13$ ,  $P = 0.000$  and  $P = 0.000$ , respectively) and corresponding endosperm diets, HOE1 ( $P = 0.001$ ,  $P = 0.000$  and  $P = 0.000$ , respectively), HOE2 ( $P = 0.002$ ,  $P = 0.000$  and  $P = 0.000$ , respectively), and OE2 ( $P = 0.009$ ,  $P = 0.006$  and  $P = 0.018$ , respectively). Increased concentrations of saponifiable EDCA and DCA were found in the animals fed on the RB diet compared with the FF diet (Table 8). The concentrations of free LCA in the animals fed on oat-bran diets (HOB and OB) were unchanged compared with those for the animals fed on the FF diet while the concentration of free LCA in the animals fed on the RB diet was significantly lower than that for the animals fed on the FF ( $P = 0.002$ ) and RE ( $P = 0.003$ ) diets (Table 9).

A scatter map (Fig. 1) shows the concentration of biliary LCA *v.* daily excretion of faecal saponifiable LCA. In the bran groups (HOB, OB and RB) the concentration of biliary LCA was always low ( $\leq 0.99$  mmol/l, which is the mean value for the endosperm and FF dietary groups). In the endosperm and FF dietary groups the faecal saponifiable LCA was always low ( $\leq 74.9$  nmol/d, which is the mean value of the bran dietary groups).

#### LCA:DCA in the bile and in the faeces

LCD:DCA in the bile of the OB group was lower than that for the FF dietary group ( $P = 0.035$ ), and that for the RB group was lower than those for the RE ( $P = 0.038$ ) and FF ( $P = 0.010$ ) dietary groups (Table 6).

Total LCA (LCA + epi-LCA):total DCA (DCA + epi-DCA) in the faeces of the bran-

Table 8. *Effect of oat and rye fractions on concentration of individual saponifiable bile acids in faeces of Syrian golden hamsters (Mesocricetus auratus)*

(Mean values and standard deviations for thirteen hamsters/group)

Dietary group‡	Concentration of faecal saponifiable bile acid fractions (nmol/g)											
	Epi-lithocholic		Lithocholic		Epi-deoxycholic		Deoxycholic		Chenodeoxycholic		Cholic	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HOB	17	7	128*	55	25*	15	197*	108	19	8	19*	14
HOE1	8*†	8	64†	28	4†	4	51†	25	7†	7	2†	5
HOE2	10†	6	69†	21	6†	6	58†	31	14	11	0†	0
OB	13	5	104*	41	20*	12	162*	84	18	7	23*	11
OE2	9	6	66†	26	8†	7	91*†	56	11	8	5†	7
RB	10	5	82	39	15*	16	114*	66	13	6	13*	14
RE	9*	3	67	30	7	6	69	53	12	15	5	7
FF	14	8	65	32	4	6	43	26	12	11	3	6

HOB, heat-treated oat bran; HOE1, HOE2, heat-treated oat outer and inner endosperm respectively; OB, oat bran; OE2, oat inner endosperm; RB, rye bran; RE, rye endosperm; FF, fibre-free.

Mean values were significantly different from those for the FF dietary group: \*  $P < 0.05$ .

Mean values were significantly different from those for the corresponding bran dietary group: †  $P < 0.05$ .

‡ For details of composition, see Tables 1–3.

Table 9. *Effect of oat and rye fractions on concentration of individual free bile acids in faeces of Syrian golden hamsters (Mesocricetus auratus)*

(Mean values and standard deviations for thirteen hamsters/group)

Dietary group‡	Concentration of faecal free bile acid fractions (nmol/g)											
	Epi-lithocholic		Lithocholic		Epi-deoxycholic		Deoxycholic		Chenodeoxycholic		Cholic	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HOB	217	42	1378	254	128*	45	1233*	368	58	25	71	78
HOE1	240	96	1416	450	75†	40	789*†	223	62	26	46	49
HOE2	215	50	1309	339	80†	54	773*†	363	55	27	51	56
OB	183*	36	1129	242	109*	52	1085*	453	63	27	68	61
OE2	248†	73	1555†	474	107	43	1248*	470	57	14	29	39
RB	135*	45	849*	284	91*	56	731*	409	56	37	62	58
RE	213†	47	1229†	312	62	49	634	387	55	16	48	41
FF	299	157	1279	336	60	43	396	199	70	18	39	44

HOB, heat-treated oat bran; HOE1, HOE2, heat-treated oat outer and inner endosperm; OB, oat bran; OE2, oat inner endosperm; RB, rye bran; RE, rye endosperm; FF, fibre-free.

Mean values were significantly different from those for the FF dietary group: \*  $P < 0.05$ .

Mean values were significantly different from those for the corresponding bran dietary group: †  $P < 0.05$ .

‡ For details of procedures, see Tables 1–3.

supplemented groups (mean and SD): 1.20 (SD 0.46) for HOB, 1.21 (SD 0.61) for OB and 1.43 (SD 1.06) for RB, were significantly lower than those for the corresponding endosperm-supplemented dietary groups: 1.93 (SD 0.51) for HOE1 ( $P = 0.001$ ), 2.05 (SD 0.74) for HOE2 ( $P = 0.002$ ) and 2.35 (SD 0.75) for RE ( $P = 0.017$ ), and the FF dietary group 3.67 (SD 1.08)



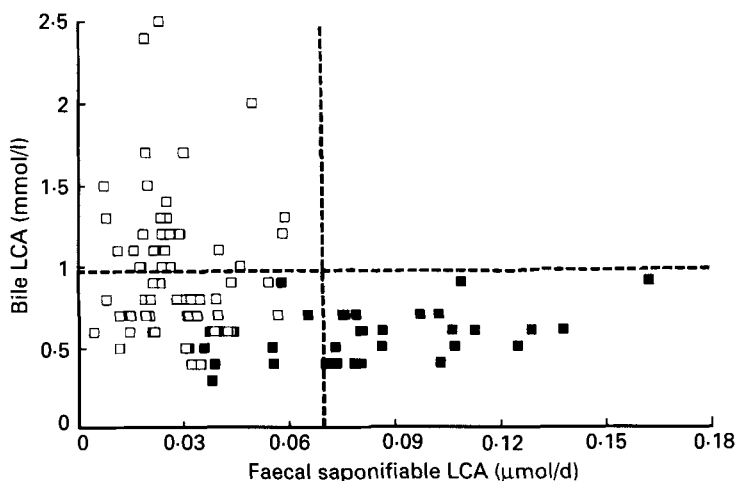


Fig. 1. Scatter map of the concentration of biliary lithocholic acid (LCA) v daily excretion of faecal saponifiable LCA for all experimental groups of Syrian golden hamsters (*Mesocricetus auratus*) fed on oat and rye fractions. (□), low-fibre and fibre-free dietary groups; (■), high-fibre dietary groups. (---), The mean concentration of biliary LCA in the low-fibre dietary groups; (:) the mean daily excretion of faecal saponifiable LCA in the high-fibre dietary groups. For details of diets and their composition, see Tables 1-3.

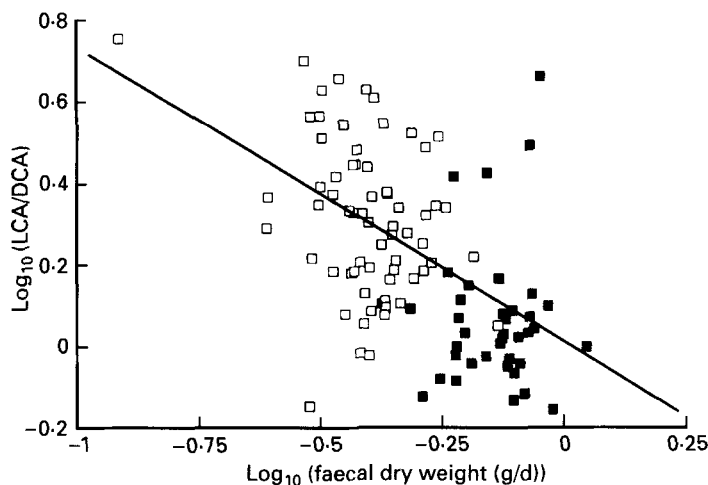


Fig. 2. The relationship between  $\log_{10}$  total lithocholic acid (LCA):total deoxycholic acid (DCA) and  $\log_{10}$  faecal dry weight in faeces from Syrian golden hamsters (*Mesocricetus auratus*) fed on oat and rye fractions (□), low-fibre and fibre-free dietary groups; (■), high-fibre dietary groups. For details of diets and their composition, see Tables 1-3.

( $P = 0.000$  respectively). However, there was no difference between the OB and OE2 1.32 (SD 0.23) dietary groups. The ratios for all endosperm-supplemented groups were also significantly lower than those for the FF dietary groups (HOE1, HOE2 and OE2  $P = 0.000$ ; RE  $P = 0.001$ ).

LCA:DCA for bile was positively correlated with that for faeces ( $r = 0.33$ ,  $P < 0.001$ ,  $n = 104$ ). The faecal  $\log_{10}$  total LCA: total DCA was negatively well correlated with  $\log_{10}$  faecal dry weight (Fig. 2;  $r = -0.54$ ,  $P < 0.001$ ,  $n = 104$ ).

## DISCUSSION

Oats have recently received increased attention from the nutrition and medical communities because of their dietary fibre content and role in reducing blood cholesterol levels, a recognized risk factor for coronary heart disease. Although the mechanism(s) for the hypocholesterolaemic effect of oats is not clear, an increase in faecal excretion of bile acids (Judd & Truswell, 1981; Illman & Topping, 1985; Zhang *et al.* 1992) by oat fibre, mainly the mixed-linked  $\beta$ -glucans, is supposed to contribute to the effect (Henry, 1987; Shinnick *et al.* 1988).

Attention has also been paid to the increased faecal excretion of bile acids after intake of oats, because bile acids and their derivatives from bacterial fermentation have been suggested to act as carcinogens (Turjman *et al.* 1984; Owen *et al.* 1987; Nair, 1988). An increased proportion of secondary bile acids might increase the risk of colo-rectal cancer (Mullan *et al.* 1990) and faecal LCA:DCA may be an important aetiological factor in colo-rectal cancer (Owen *et al.* 1983).

The present study shows that the bran diets increased both the daily faecal excretion of bile acids and faecal weight. As a result of the dilution effect of the increased faecal weight induced by the different bran diets, the concentrations of TBA were unchanged in the animals fed on the RB diet (Table 7). The concentration of FBA was unchanged in the animals fed on unsteamed flaked oat-bran and RB diets. The concentration of free LCA and ELCA was reduced in the animals fed on unsteamed flaked oat-bran and RB diets (Table 9), while the bran groups had an increased concentration of free DCA and EDCA compared with the groups fed on the FF diet. The difference in concentrations of faecal free LCA and FBA between the animals fed on steamed flaked and unsteamed flaked oat-bran diets suggested that the altered physical state of oats produced by heating may have slightly changed the physiological effects. Dietary fibre, its dilution effects and binding of bile acids have been suggested to prevent the carcinogenesis of the colon (Jenkins *et al.* 1986; Kritchevsky, 1986; Greenwald *et al.* 1987).

The conjugated bile acids secreted from the liver undergo deconjugation when passing to the large intestine. Part of the deconjugated bile acids, free bile acids, are esterified in the large intestine to form saponifiable derivatives, for example  $C_{24}$  carboxyl esters or fatty acyl esters (Norman, 1964; Kelsey & Sexton, 1976). The biological importance of the SpBA is not clear. The present experiment showed that both oat and rye bran increased the daily faecal excretion and the concentration of faecal SpBA. The concentration of biliary LCA v. the daily excretion of faecal saponifiable LCA from all individual animals is shown in Fig. 1. When the high-fibre dietary groups were separated from the low-fibre groups in the scatter map the concentrations of biliary LCA in all the animals in the HFD groups were low, while the daily excretion of saponifiable LCA in all the animals in the LFD or FF groups was low. The results strongly suggest that the absorption of LCA from the large intestine, which is related to the biliary concentration of LCA, was directly associated with the fibre content in the diet, and that the esterification of bile acids was also influenced by the dietary fibre. The free secondary bile acids, especially free LCA, are thought to be toxic and carcinogenic to the colonic mucosa (Rafter *et al.* 1987), and thus the colonic mucosa of many animals in the LFD groups is obviously more exposed to the free LCA. The SpBA fraction accounts for 5–10% of the total bile acids. The characteristics and the biological importance of this bile acid fraction should be studied further.

The faecal LCA:DCA values for both bran- and endosperm-supplemented dietary groups are much lower than that for the FF dietary group. The lowest faecal LCA:DCA values were obtained with the bran-supplemented HFD groups. The correlation between the biliary and faecal LCA:DCA values indicated the logical relationship between the

faecal and biliary bile acids. LCA:DCA has been suggested to be an indicator for the risk of colo-rectal cancer in humans (Owen *et al.* 1983). It has been observed in humans that there is a significant regression for  $\log_{10}$  LCA:DCA *v.*  $\log_{10}$  stool weight (Brydon *et al.* 1988). In the present animal experiment we observed a similar result;  $\log_{10}$  LCA(total):DCA(total) was well correlated with  $\log_{10}$  faecal dry weight (Fig. 2). The results from HFD groups were located at the lower-right section of the map with a few exceptions, while the results from LFD groups were mostly found at the upper-left section of the map.

In conclusion, oat and rye bran intake increased the faecal weight and the daily excretion of total bile acids in hamsters. Oat and rye bran intake reduced the concentration of LCA in the bile. The reduced concentration of biliary LCA could be related to a decreased concentration of free LCA in the large intestine. Both bran and endosperm diets reduced the faecal LCA:DCA value compared with a fibre-free diet.

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