Effect of oat saponins on plasma and liver lipids in gerbils (*Meriones unguiculatus*) and rats

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The effects of oat saponins (a mixture of avenacosides A and B) on plasma and liver lipids in gerbils (*Meriones unguiculatus*) and rats were investigated. Cholesterol-containing diets high in total and saturated fat and with different avenacoside contents (zero (ethanol-extracted oats), normal (oats) and twice normal (ethanol-extracted oats plus added avenacosides)) were used. Compared with a cellulose control group the oat diets in both species gave a significantly higher cholesterol content in the HDL fraction and a significantly lower liver cholesterol content. No significant differences in total plasma cholesterol, HDL-cholesterol and plasma triacylglycerols were found, however, between the groups fed on oats with different avenacoside content. The liver weight, total liver cholesterol and free liver cholesterol were also similar, whereas the liver lipid content was significantly lower in rats given the highest amount of avenacosides compared with zero or normal amounts. The tendency was the same in gerbils. Thus, the oat saponins had only minor effects on lipid metabolism in gerbils and rats.

Gerbil: Lipid metabolism: Oats: Saponins

Oats have well-documented blood-cholesterol-lowering effects (Ripsin & Keenan, 1992). There is evidence that the soluble fibre in oats, β-glucan, is responsible for this effect (Chen *et al.* 1981; Shinnick *et al.* 1990). In rats given atherogenic diets a dose–response relationship has been demonstrated between the β-glucan content and the lipid-lowering effect. The average content of β-glucan in oats is about 50 g/kg (Asp *et al.* 1991). The original mechanism proposed for the action of soluble fibre, i.e. increased ileal sequestration of bile acids, has been confirmed in both animals and man (Illman & Topping, 1985; Andersson, 1992). The propionate formed when the fibre is fermented in the colon could also contribute by inhibiting cholesterol biosynthesis (Chen & Anderson, 1984).

Another constituent in oats which could affect the cholesterol concentration in blood is the saponins. Saponins are glycosides with steroid or triterpenoid aglycon (Price *et al.* 1987). A hypothesis that saponins induce the adsorption of bile acids to dietary fibre has been proposed (Oakenfull *et al.* 1979). Bile acid excretion is increased and the blood cholesterol level reduced when rats, pigs and humans are given saponin-rich diets (Potter *et al.* 1980; Topping *et al.* 1980; Oakenfull *et al.* 1984). This hypothesis was not supported, however, by findings of a rat study with lucerne (*Medicago sativa*) and saponin-free lucerne (Story *et al.* 1984). The latter diet increased bile acid excretion more than the former.

The main saponins in oats are avenacosides A and B (Tschesche *et al.* 1969; Tschesche & Lauren, 1971). So far, there have been no studies which have investigated the cholesterol-lowering effect of these oat saponins.

Gerbils (*Meriones unguiculatus*), compared with rats, react more like humans to fat in the diet, i.e. saturated fat increases blood cholesterol (Nicolosi *et al.* 1981). On the other hand, gerbils are more sensitive to dietary cholesterol than man. They do not develop

atherosclerosis (Gordon & Cekleniak, 1961) but a diet containing 2 g cholesterol/kg causes severe hypercholesterolaemia and high levels of cholesteryl esters in the liver, ultimately leading to cirrhosis (Temmerman *et al.* 1988). In rats, 5–10 g cholesterol/kg diet, and often also bile acids, must be added to produce elevated blood cholesterol levels (Shinnick *et al.* 1990).

In the present study we fed gerbils on diets containing 1 g cholesterol/kg and 40 % of the energy from fat, i.e. similar to Western diets. For the rats the cholesterol content was increased to 5 g/kg diet with the same fat content. The experimental diets contained either oats, ethanol-extracted oats in which most of the saponins had been removed, or ethanol-extracted oats plus added avenacosides (0·7 g/kg diet). In this way oat diets with three levels of avenacosides (zero, normal, twice normal) were prepared. As controls we used one diet with cellulose, known not to affect blood cholesterol, and another with guar gum, known to reduce hypercholesterolaemia in rats. To our knowledge gerbils have not been used previously to study the effects of dietary fibre on plasma lipid levels.

MATERIALS AND METHODS

Preparation and analysis of oatmeals

Oatmeal of the variety Sang was obtained from Kungsörnen, Järna, Sweden. A portion of the oatmeal was extracted with ethanol to obtain a virtually saponin-free product. This procedure was carried out by Swedish Protein, Väröbacka, Sweden. The meal was extracted twice at room temperature with 700 ml ethanol/l (1:4, w/v). After this treatment 8 % of the avenacosides remained, therefore, the meal was extracted a third time, now with ethanol (950 ml/l) at 55–60° for 24 h. This meal, containing about 2 % of the initial amount of avenacosides (Table 1), was used in the study.

The oatmeal and the ethanol-extracted oatmeal were analysed for avenacosides A and B using HPLC technique (Önning & Asp, 1993). The meal was first defatted with light petroleum (b.p. 60–80°) and, thereafter, the saponins were extracted with methanol. A portion of the extract (20 μl) was injected onto an octyl silica column (LiChroCART, 125 × 4 mm; Merck, Darmstadt, Germany) and the saponins were eluted with a gradient of 250–400 ml acetonitrile/l for 15 min. A Varian 5000 Liquid Chromatograph was used and the flow-rate was 2 ml/min. The saponins were detected by u.v. absorption at 200 nm and calibration was performed by injecting known amounts of avenacosides A and B. The avenacoside A and B standards used were prepared from oatmeal (Önning *et al.* 1994).

The meals were analysed for dietary fibre (Asp *et al.* 1983), β-glucan (McCleary & Glennie-Holmes, 1985) and starch (Holm *et al.* 1986) using enzymic methods. Protein was analysed by the Kjeldahl procedure and tocopherols and tocotrienols using an HPLC technique (Bourgeois *et al.* 1985; Håkansson *et al.* 1987). Fat (EU Method, 1971) and fatty acid (Berg, 1990) analyses were performed at the Swedish Meat Research Institute, Kävlinge, Sweden.

Isolation of avenacosides

Avenacosides A and B were extracted from 20 kg oatmeal (var. Sang, Kungsörnen, Järna, Sweden) at Nestlé Research Centre, Lausanne, Switzerland. The meal was first defatted with hexane and, thereafter extracted with methanol at 25°. The methanol extract was fractionated by portioning into butanol–water (1:1, v/v). The phases were allowed to separate and the butanol phase containing the saponins was evaporated to dryness under reduced pressure. The residue was purified using chromatography on silica gel SI-60. Elution was carried out using a stepwise gradient with chloroform and increasing amounts of methanol (0–250 ml methanol/l). The fractions containing saponins, confirmed by thin-layer chromatographic analysis (Lütz, 1980), were pooled and concentrated. The resulting saponin material was further purified by gel filtration (Sephadex LH-20) with ethanol as the

Table 1. *Nutrient content in oatmeal and ethanol-extracted oatmeal* (g/kg dry matter)*
(Values are means for two or three analyses)

Nutrient	Oatmeal	EtOH-extracted oatmeal
Dietary fibre		
Total	97	118
Insoluble	49	58
Soluble	48	59
β-Glucan	49	62
Starch	645	668
Protein (N × 5·83)	131	144
Fat		
Total	78	12
Fatty acid composition (g/100 g)		
C_6–C_{12}	<0·1	<0·1
C_{14}	0·3	0·5
C_{16}	16·9	26·5
$C_{16:1}$	0·2	0·3
C_{18}	1·5	1·6
$C_{18:1}$	35·3	27·2
$C_{18:2}$	40·2	34·4
$C_{18:3}$	1·3	0·8
C_{20}	0·2	0·5
$C_{20:1}$	0·7	<0·1
C_{22}	0·3	0·8
$C_{22:1}$	0·1	3·0
C_{24}	0·1	<0·1
$C_{24:1}$	0·1	<0·1
Saponins		
Avenacoside A	0·30	0·007
Avenacoside B	0·19	0·006
α-Tocopherol	0·0022	<0·0001

* For details of extraction procedure, see p. 276.

eluting solvent. In the next purification step a column was filled with RP-C_{18} and equilibrated in 600 ml methanol/l. The saponin sample was loaded onto the column which was eluted first with 600 ml methanol/l and then 800 ml methanol/l. The saponin-containing fractions were pooled and evaporated to dryness, and about 4 g of a yellow-coloured powder were obtained after this procedure. The contents of avenacosides A and B were 0·52 and 0·09 g/g dry matter respectively. Final purification was achieved using Bond Elut cartridges (10 g, C_{18}; Analytichem International, Varian, Harbour City, CA, USA) according to the method of Önning & Asp (1993). The resulting material was slightly yellow and contained 0·69 g avenacoside A and 0·14 g avenacoside B/g dry matter. This preparation was added to the ethanol-extracted meal to obtain an avenacoside content (1 g/kg) which was about twice that in the original meal.

Preparation of diets

The experimental diets, except the rat-pellet diet, were balanced regarding dietary fibre, carbohydrate, protein and fat (Table 2). The gerbil and rat diets were the same in all respects except for the cholesterol content. The main components were cellulose (Whatman, Maidstone, Kent), guar gum (Copenhagen Pectin Factory Ltd, Skensved, Denmark), wheat starch (Excelsior, Holland), sucrose (Swedish Sugar Company, Arlöv, Sweden), casein (ICN Biochemicals, Cleveland, OH, USA) and a fat mixture containing coconut oil, palm oil and rapeseed oil (Karlshamns Oils & Fats AB, Karlshamn, Sweden). Other

Table 2. *Composition of the experimental diets (g/kg dry matter)*

Ingredient	Cellulose	Guar gum	Ethanol-extracted oatmeal‡	Oatmeal	Ethanol-extracted oatmeal + saponins
Dietary fibre					
Total	65	65	65	65	65
Cellulose	65	—	—	—	—
Guar gum	—	65	—	—	—
Oatmeal	—	—	65	65	65
Carbohydrate					
Total	520	520	520	520	520
Sucrose	90	90	90	90	90
Wheat starch	430	430	60	—	60
Oatmeal	—	—	370	430	370
Protein					
Total	150	150	150	150	150
Casein	150	150	70	60	70
Oatmeal	—	—	80	90	80
L-methionine	1	1	1	1	1
Taurine	5	5	5	5	5
Fat					
Total	200	200	200	200	200
Coconut oil	100	100	100	100	100
Palm oil	50	50	50	50	50
Oatmeal	—	—	8	50	8
Fat mix*	50	50	42	—	42
Mineral mix†	48	48	48	48	48
Vitamin mix†	8	8	8	8	8
Choline chloride	2	2	2	2	2
Cholesterol					
Gerbil	1	1	1	1	1
Rat	5	5	5	5	5
Avenacosides	—	—	—	0·35	0·70

* The fat mix was added to balance the oatmeal fat and consisted of (g/kg): palm oil 260, rapeseed oil 150, maize oil 590.

† For details of composition, see Nyman *et al.* (1990).

‡ For details of extraction procedure, see p. 276.

ingredients in the diets were L-methionine (Merck), taurine (Sigma Chemical Company, St Louis, MO, USA), mineral and vitamin mix (Hospital Pharmacy, Lund, Sweden), and choline chloride (Kebo Lab AB, Stockholm, Sweden). The gerbil and rat diets contained 1 and 5 g cholesterol (crystalline cholesterol; Merck)/kg respectively. In these diets 40% of the energy comes from fat, 47% from carbohydrate and 13% from protein. The fats were used in proportions adjusted to achieve a similar fatty acid composition in all the diets (Table 3).

The rat-pellet diet (R3; Lactamin, Stockholm, Sweden) contained (g/kg dry matter): protein 240, fat 60, crude fibre 40, carbohydrate 590. Analysis of dietary fibre (Asp *et al.* 1983) gave a total content of 171 g/kg: 140 g insoluble and 31 g soluble fibre.

Experimental design

Male gerbils (Centre d'Elevage R. Janvier, Le-Genest-St-Isle, France) and male rats (Sprague–Dawley; B&K Universal AB, Sollentuna, Sweden) were used. The gerbils were divided into five groups (fourteen in each group) with initial mean weights of 51 (SD 3) g and housed two per stainless-steel cage. The rats were housed individually and six groups

Table 3. *Fatty acid composition (g/100 g fatty acids) of the experimental diets**

Fatty acid	Cellulose, guar gum	Oatmeal	Ethanol-extracted oatmeal†
C_6	0·3	0·3	0·3
C_8	3·8	3·8	3·8
C_{10}	2·8	2·8	2·8
C_{12}	22·3	22·3	22·3
C_{14}	9·4	9·4	9·4
C_{16}	20·3	19·9	22·4
$C_{16:1}$	0·1	0·1	0·1
C_{18}	3·1	2·9	2·9
$C_{18:1}$	22·8	23·0	20·8
$C_{18:2}$	14·1	13·9	12·3
$C_{18:3}$	0·6	0·4	0·3
C_{20}	0·2	0·2	0·3
$C_{20:1}$	0·2	0·3	0·1
C_{22}	0·1	0·1	0·2
$C_{22:1}$	—	—	0·8
C_{24}	0·1	—	—
$C_{24:1}$	—	—	—
Total saturated fatty acids	62·4	61·7	64·4
P:S	0·24	0·23	0·20

P:S, polyunsaturated fatty acids: saturated fatty acids.
* For details of composition, see Tables 1 and 2.
† For details of extraction procedure, see p. 276.

of rats with a mean initial weight of 152 (SD 5) g were selected. Each group consisted of eight animals, except for the group fed on the diet with added saponins in which there were only five animals due to the limited amount of saponins available. The animals were kept in air-conditioned rooms, at 25° and 50% relative humidity, with free access to water. A 12 h light–dark cycle was used.

The different diets were fed *ad lib.* in metal containers designed to give minimal losses. Feed consumption was registered every second day and the weight of the animals weekly. The animals were acclimatized on pellets for 2 d. One group of rats remained on this diet as a control, while the rest were transferred to the test diets. The gerbils were fed on their diets for 21 d, except for those given the cellulose diet which was discontinued after 16 d. The rats were given the diets for 19 d.

After the feeding period the animals were fasted overnight and killed by CO_2 narcosis. Blood was withdrawn by cardiac puncture into tubes containing sodium EDTA as an anticoagulant. The plasma was separated by centrifugation at 10000 ***g***, for 5 min, then the plasma samples were refrigerated. The liver was excised, rinsed in NaCl solution (9 g/l distilled water), blotted dry, weighed and frozen in NaCl solution.

The study was approved by the Ethical Committee for Animal Studies at the University of Lund.

Analysis of lipids

The plasma samples were analysed within 24 h for total cholesterol, HDL-cholesterol and triacylglycerols using a Reflotron instrument (Boehringer-Mannheim). The apparatus was standardized throughout the analysis using control serum: Precinorm U (total cholesterol, triacylglycerols) and Precinorm HDL. The coefficients of variation for total cholesterol, HDL-cholesterol and triacylglycerol determinations were 1·4, 4·5 and 3·3% respectively. The cholesterol content in the plasma from the rats was below the detection limit

(< 2·59 mmol/l) and, thus, the plasma content was determined using the Sigma cholesterol diagnostic kit (Sigma chemical Co., St Louis, MO, USA). The two procedures gave almost identical results when tested on rat plasma containing 2·7 and 3·1 mmol total cholesterol/l respectively. Non-HDL-cholesterol, representing mainly the LDL-cholesterol, was calculated as total minus HDL-cholesterol.

Liver lipids were extracted with a chloroform–methanol mixture using the method of Folch *et al.* (1956). Total liver lipid content was determined gravimetrically. The Sigma diagnostic kit was used to determine the total liver cholesterol. A detergent, Triton X-100 (2·5 mg), was added to the extract and the solution was evaporated under N_2 gas before the analysis (Carlson & Goldfarb, 1977). Free liver cholesterol was determined with the Boehringer Mannheim cholesterol test (Boehringer Mannheim Scandinavia AB, Bromma, Sweden).

All analyses were performed at least in duplicate.

Statistical evaluation

The SPSS computer program was used and group means were compared by one-way analysis of variance followed by Duncan's multiple-range test. Values for $P < 0·05$ were considered significantly different. For total liver cholesterol (rats) the standard deviation was approximately proportional to the means and, therefore, a logarithmic transformation was used to stabilize the variance in this case. For final body weight and feed intake there were significant differences in variances between the dietary groups, and the *t* test for independent samples was used. Plasma triacylglycerol values followed a skewed distribution and the non-parametric Kruskal–Wallis test was used.

RESULTS

Nutrient content in the oatmeal and the ethanol-extracted oatmeal

Analytical results are presented in Table 1. Avenacoside A and B contents were reduced from 0·49 to 0·013 g/kg dry matter by ethanol extraction and large amounts of fats (85%) were also extracted by the ethanol. α-Tocopherol could be detected in the untreated meal but not in the extracted meal. Tocotrienols may affect the blood cholesterol level (Querishi *et al.* 1986) but neither of the meals contained any detectable tocotrienols. The other nutrients (dietary fibre, starch, protein) were affected to a minor extent; there was a slight increase in dietary fibre content and, calculated for fat-free material, a slight reduction in starch content after ethanol extraction. This indicates the formation of some resistant starch by the extraction process.

Growth and feed intake

There were no significant differences in initial weight between the groups of either gerbils or rats (Table 4). Due to several spontaneous deaths in the gerbil group fed on cellulose, this group was withdrawn after 16 d. The other groups were fed for 21 d (gerbils) and 19 d (rats). For both species the intake of the guar-gum diet was lower than that of the other diets and, consequently, the mean final body weight for this group was significantly lower. The intake of the cellulose diet was also lower in rats, especially at the beginning of the trial (values not shown). The groups fed on diets containing oats with different saponin contents had similar feed intakes and final body weights.

Plasma lipids

The plasma lipid values are presented in Table 5. The total plasma cholesterol contents for the gerbils were between 4·3 and 5·1 mmol/l and were not significantly different between the dietary groups. For the rats the guar-gum group had a total plasma cholesterol level of

Table 4. *The effects of oat saponins on the growth and feed intake of gerbils* (Meriones unguiculatus) *and rats given different diets**

(Mean values with their standard errors)

Diet	*n*	Initial body wt (g) Mean	SE	Final body wt† (g) Mean	SE	Intake (g/d per animal) Mean	SE
Gerbils†							
Cellulose	7	51·9[a]	0·9	55·5	1·1	4·4[a]	0·2
Guar gum	13	50·9[a]	1·2	51·7[b]	2·6	3·9[b]	0·1
EtOH oats	14	51·5[a]	1·0	59·4[a]	1·0	4·8[a]	0·02
Oats	14	51·5[a]	0·6	59·7[a]	1·1	4·8[a]	0·05
EtOH oats + saponins	14	51·1[a]	0·7	58·5[a]	1·2	4·8[a]	0·1
Rats†							
Pellets	8	151·9[a]	2·1	265·5[bc]	6·1	17·3[a]	0·4
Cellulose	8	152·7[a]	2·0	259·7[ac]	11·9	14·3[c]	1·0
Guar gum	8	152·4[a]	1·7	220·0[d]	3·7	10·9[d]	0·3
EtOH oats	8	151·7[a]	1·1	282·0[a]	3·4	16·6[ac]	0·4
Oats	8	152·3[a]	1·6	274·4[ac]	6·2	15·6[bc]	0·5
EtOH oats + saponins	5	151·6[a]	3·0	270·0[ac]	5·0	16·0[bc]	0·3

[a–d] Means within columns with the same superscript letter were not significantly different ($P > 0·05$).
EtOH oats, ethanol-extracted oats.
* For details of animals and diets, see Tables 1–3 and pp. 276–278.
† Values for gerbils after 21 d on the diet except for the cellulose diet which was fed for 16 d. The rats were fed for 19 d.

Table 5. *The effects of oat saponins on plasma lipid concentrations (mmol/l) for gerbils* (Meriones unguiculatus) *and rats given different diets**

(Mean values with their standard errors)

Diet	Total cholesterol Mean	SE	HDL-cholesterol Mean	SE	Non-HDL-cholesterol Mean	SE	Triacylglycerols Mean	SE
Gerbils†								
Cellulose	5·0[a]	1·5	1·9[a]	0·2	3·1[a]	0·6	0·9[a]	0·06
Guar gum	4·3[a]	1·1	2·3[b]	0·2	1·9[b]	0·2	1·3[a]	0·1
EtOH oats	4·8[a]	1·0	2·5[b]	0·1	2·3[b]	0·1	1·2[a]	0·2
Oats	5·1[a]	1·0	2·6[b]	0·1	2·5[ab]	0·2	1·0[a]	0·05
EtOH oats + saponins	4·7[a]	1·0	2·5[b]	0·1	2·2[b]	0·2	1·1[a]	0·1
Rats†								
Pellets	1·9[b]	0·1	1·1[bc]	0·1	0·9[bc]	0·06	0·8[a]	0·05
Cellulose	2·0[b]	0·1	0·9[c]	0·06	1·1[ac]	0·08	0·8[a]	0·07
Guar gum	2·5[a]	0·1	1·3[a]	0·07	1·2[a]	0·10	1·1[a]	0·08
EtOH oats	2·1[b]	0·1	1·3[a]	0·1	0·8[b]	0·07	0·9[a]	0·05
Oats	2·1[ab]	0·1	1·2[a]	0·09	0·9[bc]	0·04	0·9[a]	0·08
EtOH oats + saponins	2·1[b]	0·04	1·1[ac]	0·04	1·0[abc]	0·06	1·0[a]	0·04

[a–c] Means within columns with the same superscript letter were not significantly different ($P > 0·05$).
EtOH oats, ethanol-extracted oats.
* For details of animals and diets, see Tables 1–3 and pp. 276–278.
† The feeding period was 21 d for gerbils, except for the cellulose diet which was fed for 16 d, and 19 d for rats.

Table 6. *The effects of oat saponins on liver weight and liver lipid values for gerbils* (Meriones unguiculatus) *and rats given different diets**

(Mean values with their standard errors)

	Liver wt				Total lipid content (mg/g wet tissue)		Cholesterol (mg/g wet tissue)			
	g		g/kg body wt				Total		Free	
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Gerbils†										
Cellulose	1·9^{a}	0·08	34^{a}	1·0	117^{a}	10·2	10·4^{a}	0·6	2·0^{a}	0·05
Guar gum	1·9^{a}	0·09	37^{a}	0·5	123^{a}	8·0	5·5^{b}	0·5	1·8^{b}	0·04
EtOH oats	2·1^{a}	0·06	35^{a}	0·7	116^{a}	6·9	6·9^{c}	0·2	1·7^{b}	0·04
Oats	2·1^{a}	0·06	34^{a}	0·8	115^{a}	6·4	6·5^{bc}	0·3	1·8^{b}	0·04
EtOH oats + saponins	2·0^{a}	0·05	35^{a}	0·8	111^{a}	4·8	6·5^{c}	0·3	1·6^{b}	0·04
Rats†										
Pellets	9·9^{b}	0·5	37^{b}	1	50^{c}	2·8	3·8^{c}	0·3	1·8^{c}	0·08
Cellulose	11·2^{ab}	0·6	43^{a}	1	163^{a}	11·1	25·8^{a}	1·5	2·1^{a}	0·10
Guar gum	8·9^{c}	0·3	40^{ab}	1	99^{b}	7·5	13·0^{b}	1·2	2·1^{a}	0·05
EtOH oats	11·2^{ab}	0·4	40^{ab}	1	146^{a}	9·9	11·5^{b}	0·7	1·6^{c}	0·10
Oats	11·6^{a}	0·5	42^{a}	1	150^{a}	13·5	12·3^{b}	0·7	1·9^{b}	0·10
EtOH oats + saponins	10·6^{ab}	0·4	39^{ab}	2	101^{b}	18·1	11·7^{b}	1·5	1·7^{c}	0·10

$^{a-c}$ Means within columns with the same superscript letter were not significantly different ($P > 0·05$).
EtOH oats, ethanol-extracted oats.
* For details of animals and diets, see Tables 1–3 and pp. 276–278.
† The feeding period was 21 d for gerbils, except for the cellulose diet which was fed for 16 d, and 19 d for rats.

2·5 mmol/l, which was significantly higher than that of the other groups which was about 2 mmol/l. The cholesterol content of the HDL fraction was significantly lower for the cellulose group than those for all other groups for gerbils and lower than those for the guar-gum, ethanol-extracted and normal oat groups in rats. The HDL-cholesterol values for the different oat groups were similar, regardless of the saponin content, about 2·5 mmol/l for the gerbils and about 1·2 mmol/l for the rats. For the gerbils, non-HDL-cholesterol values were lowest for the guar-gum group, intermediate for the oat groups and highest for the cellulose group. The rats had lower values which tended to be similar, ranging from 0·8 to 1·2 mmol/l. The plasma triacylglycerol levels were not significantly different between the dietary groups.

Liver weight and lipid content

The liver weight was about 2 g for the gerbils and similar in all groups (Table 6). No differences were found in relative liver weights. For the rats the liver weights differed significantly and were lowest for the guar-gum and rat-pellet groups. The significant difference remained for the rat-pellet group but not for the guar-gum group when the liver weight was expressed as g/kg body weight. The total lipid content was similar in the different groups of gerbils, but significantly lower in rats fed on pellets or guar gum than in those fed on cellulose, extracted oatmeal or unextracted oatmeal. There was also a significantly lower content of total liver lipids in the rats fed on the diet with added saponins, than in the oatmeal groups that were not supplemented with saponins.

Table 6 shows that there were pronounced differences in total liver cholesterol. For both species the cellulose group exhibited about twice the amount of the oatmeal groups; means for the gerbils were 10·4 *v.* 6·6 mg/g liver respectively and for the rats were 25·8 *v.* 11·8 mg/g liver respectively. The rat-pellet group had the lowest value (3·8 mg/g liver) and this was

significantly lower than those for the other dietary groups. In the gerbils, free liver cholesterol was highest for the cellulose group. In the rat experiment the cellulose group and also the guar-gum group had a higher level of free cholesterol than the groups fed on oats.

DISCUSSION

Although the extracted oatmeal diet is referred to as having no saponins, it was not possible to obtain complete extraction of avenacosides A and B from oats. About 2% of the original amount remained, despite repeated extractions with ethanol. Compared with other studies, however, where a saponin-depleted material also was desired, this is a good result. In other studies using 800 ml ethanol/l only about 80% of the saponins were extracted (Potter *et al.* 1980; Topping *et al.* 1980; Calvert *et al.* 1982).

In some studies where saponins have been added to diets the intakes become lower, probably because of the bitter taste (Southon *et al.* 1988; Potter *et al.* 1993). Avenacosides A and B are bitter-tasting (Tschesche *et al.* 1969) but the levels used in the present study (maximum 0·7 g/kg diet) did not affect the intake compared with the other groups, for any of the species.

The mean weight gain of the gerbils was 7·8 g for the oatmeal groups, which is slightly less than that found in some previous studies (Mercer & Holub, 1981; Andersen & Holub, 1982), but similar to that in another recent study (Potter *et al.* 1993). Spontaneous deaths occurred in the cellulose group, possibly due to stress. A high mortality rate was reported in a previous study in which gerbils were fed on a 20 g cholesterol/kg diet for several months (Temmerman *et al.* 1988). For the rats the mean weight gain for the oatmeal groups was 123·6 g and this did not differ from weight gains reported in the literature (Kritchevsky *et al.* 1984).

It was very important to balance the fatty acid compositions of the diets since the blood cholesterol level of gerbils is very sensitive to fat in the diet. Gerbils given diets containing no cholesterol, with 200 g safflower oil (11 g saturated fatty acids/100 g) or 190 g beef tallow (50 g saturated fatty acids/100 g)/kg diet, exhibited blood cholesterol levels of 1·3 and 2·6 mmol/l respectively after 22 d of feeding (Leach & Holub, 1984). Hegsted & Gallagher (1967) gave diets containing 1 g cholesterol/kg to gerbils. The fats tested were safflower oil, olive oil and coconut oil (200 g/kg diet) and after 2 weeks of feeding the cholesterol levels were 4·3, 5·7 and 6·4 mmol/l respectively. We had the same cholesterol and fat levels in our diets as those used in the Hegsted & Gallagher (1967) study and obtained similar serum cholesterol levels (4·3–5·1 mmol/l).

Adding 5 g cholesterol/kg to the diet given to rats did not affect plasma lipid values substantially compared with those of the group eating rat pellets with no cholesterol. Kritchevsky *et al.* (1984) gave a cellulose or an oat-bran diet containing 5 g cholesterol/kg to rats for 21 d and, as with our findings, did not observe any differences in total plasma cholesterol. One way to obtain increased plasma cholesterol values in rats is to add cholic acid as well as cholesterol (Shinnick *et al.* 1990).

There were more pronounced effects on the plasma lipids in the gerbils and the total plasma cholesterol was higher than that in the rats, despite the lower content of cholesterol in the gerbil diets. In a comparative study the gerbils and rats exhibited plasma cholesterol levels of 7·2 and 1·7 mmol/l respectively on a diet containing 2 g cholesterol/kg (Temmerman *et al.* 1988). The higher level for the gerbils was explained as being due to the fact that gerbils could not increase faecal steroid excretion as much as the rats.

The triacylglycerol levels were similar for all groups. In a study where oat bran or oat gum was given to rats, the oat gum but not the oat bran gave decreased triacylglycerol levels (Chen *et al.* 1981).

There were profound differences in total liver lipid levels between the dietary groups in

rats and in liver cholesterol content in both species. The highest amount of saponins seemed to decrease the total liver lipid content as much as guar gum in rats, indicating some influence on the lipid metabolism in spite of the absence of effects on plasma lipids. The lowest levels of both total lipids and cholesterol in the liver were obtained in rats fed on pellets. In both species, oats as well as guar gum diminished considerably the liver cholesterol accumulation, but there was no specific effect of saponins. The total liver lipid content in our groups of rats is comparable with that of a previous study by Chen *et al.* (1981). The liver cholesterol content in the rats fed on pellets was similar (Shinnick *et al.* 1990) and that in the other groups of rats slightly higher (Kritchevsky *et al.* 1984) than that in previous studies.

The main purpose of the present study was to investigate whether saponins are involved in the hypocholesterolaemic effects of oats. The saponin levels in the diets used, therefore, were realistic compared with those found normally in oats. Earlier studies have diets with a higher saponin content. For example, Oakenfull *et al.* (1979) fed rats with a diet containing 10 g cholesterol/kg, and the addition of 10 g saponins from soapwort/kg diet lowered the cholesterol accumulation, especially in the liver. The same results were obtained with 10 g soyabean saponins/kg diet (Oakenfull *et al.* 1984). In another study rats were given soyabean flour high (22 g/kg) or low (4 g/kg) in saponins and with the same cholesterol content as in our study (5 g/kg diet; Topping *et al.* 1980). Both diets lowered plasma cholesterol, but there was no difference related to the saponin content. Adding a low amount (2 g/kg diet) of saponins from gypsophila roots to a diet also containing pectin in one study (Rotenberg & Eggum, 1986) did not reduce plasma or liver cholesterol in rats compared with the saponin-free pectin diet.

Our study has shown that saponins play no significant role in the blood hypocholesterolaemic effects of oats. Thus, other components (especially β-glucan) are responsible for the hypocholesterolaemic effects of oats. Avenacosides A and B, nevertheless, could have some minor effects, as indicated by the liver lipid contents. Lipid metabolism in the body is complex, and measuring plasma and liver lipids provides some information. To obtain a better understanding, other variables should also be investigated. There are studies which show that saponins could increase the permeability of the gut (Johnson *et al.* 1986). Our knowledge of the physiological effects of saponins is still limited and more research is needed.

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