Effect of oat saponins and different types of dietary fibre on the digestion of carbohydrates

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The effects of oat saponins (a mixture of avenacosides A and B) and dietary fibre (cellulose and guar gum) on the disaccharidase activities in the proximal small intestine of the rat were investigated. The influence of avenacosides A and B on the activity of disaccharidases and α-amylase (*EC* 3.2.1.1) was also studied *in vitro*. *In vivo*, oat diets with three avenacoside contents (negligble, normal and twice normal) were used. No significant differences in sucrase (*EC* 3.2.1.48), maltase (*EC* 3.2.1.20), trehalase (*EC* 3.2.1.28) and lactase (*EC* 3.2.1.21) activities were found between the oat groups after 19 d feeding. The rats that were given cellulose tended to have higher disaccharidase activities compared with the other groups. The avenacosides inhibited the lactase activity significantly *in vitro* while no or small effects on the other disaccharidases were found. In contrast, the *in vitro* hydrolysis of starch by α-amylase was increased in the presence of saponins, probably due to their detergent effect. Thus, the *in vitro* studies showed that the avenacosides could influence the enzyme activities. *In vivo*, these effects are probably minor due to the low avenacoside concentrations found in oats.

Oat saponins: Dietary fibre: Disaccharidases: Rat

Saponins are glycosides with a steroid or triterpene aglycone (Price *et al.* 1987). One or more sugar residues are bound to the aglycone. Saponins can be detected in some plants where they are thought to have antibiotic effects. The main property behind these effects is probably the membranolytic activity of saponins. Their primary action is to increase the permeability of a cell by combining irreversibly with discrete sites within the plasma membrane. This can eventually lead to disruption of the cell. Saponins with one sugar residue (monodesmosidic) have a higher membranolytic activity than saponins with two sugar residues (bisdesmosidic).

In oats two bisdesmosidic saponins, avenacosides A and B, have been identified (Tschesche *et al.* 1969; Tschesche & Lauren, 1971). If unheated oatmeal is mixed with water one glucose unit is split off from the avenacosides and they are converted to monodesmosides (Önning & Asp, 1993*a*). This process is probably enzymic, and since oats are normally heat-treated, mainly bisdesmosidic avenacosides can be expected to occur in oat products.

The oral toxicity of saponins is low since the saponins are probably not absorbed (Gestetner *et al.* 1968). They can, however, affect the absorption of other nutrients. For example, the absorption of cholesterol, bile acids, glucose and Fe can be inhibited by saponins (Sidhu *et al.* 1987; Southon *et al.* 1988).

Effects of food components on digestive enzymes could also influence nutrient uptake. For instance, tannic acid reduces starch digestion *in vitro* (Björck & Nyman, 1987) and dietary fibres have been shown to increase the total amount of amylase (*EC* 3.2.1.1) in the rat intestine (Schneeman & Gallaher, 1993). The activity of the small-intestinal

disaccharidases can also be influenced by food components. Thus, feeding rats with a diet containing soluble fibre increases their sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20) activities compared with rats that are fed on a fibre-free diet (Chun *et al.* 1989; Schneeman & Gallaher, 1993). Tannins have also been shown to affect disaccharidase activities (Thomsen & Tasman-Jones, 1982). The lactase (EC 3.2.1.21) activity, but not the sucrase and maltase activities, was reduced in rats given a diet containing 4 g tannins/kg.

Saponins can influence transport systems that are situated in the brush-border. For example, Sidhu *et al.* (1987) gave rats soyabean saponins (2 g/l) directly into the small intestine and this led to a reduced glucose uptake. One previous study (Kawano-Takahashi *et al.* 1986) investigated the effect of saponins on disaccharidase activities. Soyabean saponins were fed to gold-thioglucose-induced obese mice and this gave a decreased sucrase activity in the small intestine. Hence, from these few studies, saponins seem to inhibit carbohydrate digestion and absorption. The present study was carried out to investigate effects of oat saponins on the digestion of carbohydrate. Therefore, oats with different saponin contents (negligible, normal and twice normal) were fed to rats and the influence on the small-intestinal disaccharidase activities was measured. Since oats contain both soluble and insoluble dietary fibre that may also affect these enzymes, two types of purified dietary fibre (cellulose and guar gum) were fed for comparison. Finally, the effect of oat saponins, avenacosides A and B, on sucrase, maltase, trehalase (EC 3.2.1.28), lactase and α-amylase activities was studied *in vitro*.

MATERIALS AND METHODS

Animals and diets

Male rats (Sprague-Dawley, B&K Universal AB, Sollentuna, Sweden) were used. The rats were divided into six groups and given free access to the following diets: pellets (n 8), cellulose (n 8), guar gum (n 8, ethanol-extracted oats (n 8, 0·007 g avenacosides A and B/kg), oats (n 8, 0·35 g avenacosides A and B/kg) and ethanol-extracted oats plus saponins (n 5, 0·70 g avenacosides A and B/kg). The diets contained (g/kg DM): dietary fibre 65, starch 430, sucrose 90, protein 150, fat 200 and cholesterol 5 as the main ingredients. The dietary fibre in the cellulose diet was insoluble, in the guar-gum diet soluble, and in the oat diets there were about equal amounts of soluble and insoluble fibre. The rat pellet diet (R3; Lactamin, Stockholm, Sweden), fed as a reference to eight rats, contained (g/kg DM): protein 240, fat 60 and carbohydrate 590. The dietary fibre content, analysed according to Asp *et al.* (1983), was high, 171 g/kg, most of it insoluble (140 g/kg). A full description of the diets is given by Önning & Asp (1995). The mean initial weight of the rats was 152 (SD 5) g. The animals were housed individually in stainless steel cages with free access to water, at a temperature of 25°. A 12 h night and day cycle was used. Food intake was registered every second day and body weight weekly. After 19 d feeding, the animals were starved overnight and killed by CO_2 narcosis. The small intestine was removed and a 100 mm segment, 50 mm from the pylorus, was taken and frozen immediately.

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

Analysis of disaccharidase activities

The method of Dahlqvist (1968) was used. The intestinal segments were carefully homogenized in ice-cold saline (9 g NaCl/l; 25 μl/mg segment) with an Ultra-Turrax homogenizer. Diluted homogenate (40 μl) and buffer–substrate solution (40 μl) were mixed. After incubation at 37° for 60 min, the reaction was stopped by adding 1 ml TRIS-glucose oxidase (EC 1.1.3.4) reagent. Substrates used were maltose, sucrose, trehalose and lactose (56 mmol/l solutions in 0·1 M-sodium maleate buffer pH 6, making a 28 mmol/l

final substrate concentration). The colour was developed for 1 h at room temperature and the absorbance measured at 450 nm.

In separate experiments, the effect of adding saponins to the homogenate–substrate solution was tested. The final concentration of saponins was 1 or 2 mg/ml and three homogenates from the rat group eating the ethanol-extracted oat diet and three from the rat group eating the ethanol-extracted oats plus added saponins were analysed. The saponins added to the solutions were a fraction containing 0·695 g avenacoside A and 0·135 g avenacoside B/g DM, the same preparation as used to enrich the rat diet containing oats plus saponins.

The protein content of the intestinal segments was determined using the method of Lowry *et al.* (1951). Results are expressed as the amount of disaccharide (μmol/l) hydrolysed/min(= IU) per g protein.

Since some inhibition was found regarding the trehalase and lactase activities, the dependence on substrate concentration was determined with and without saponins present. The final saponin concentration was 2 mg/ml, and the final substrate concentration was varied within the following limits: trehalose, 3–56 mmol/l and lactose, 8–56 mmol/l. A homogenate containing intestines from three rats with high lactase activity was used. The results are expressed as Lineweaver–Burk plots with reaction velocity (v) as IU/ml homogenate solution.

The analyses were done at least in duplicate.

Analysis of α-amylase activity

The effect of oat saponins on α-amylase activity was studied *in vitro* according to the method of Björck & Nyman (1987). Soluble starch 'nach Zulkowsky' (Merck, Darmstadt, Germany) was incubated with α-amylase (Sigma type IA, St Louis, MO, USA) with or without saponins present. The enzyme solution was first preincubated with buffer (control) or with buffer plus oat saponins for 30 min at 37°. Final α-amylase concentrations were 0·02 or 0·2 IU/mg starch per 200 μl and final saponin concentration was 2 mg (1·7 mg avenacoside A and 0·3 mg avenacoside B)/ml. The hydrolysis was started by the addition of starch solution, and the incubation was interrupted at different time intervals with dinitrosalicylic acid (DNS); the concentration of reducing sugars was determined (Hostettler *et al.* 1951). Maltose was used as a standard and the degree of hydrolysis was calculated as the proportion (maltose equivalents) of starch degraded to maltose. When starch is hydrolysed with α-amylase different degradation products (glucose, maltose, maltotriose and limit dextrins) are formed and about 80 % hydrolysis (expressed as maltose equivalents) corresponds therefore to almost complete α-amylase degradation (Holm & Björck, 1988).

Statistical evaluation

The computer program SPSS (SPSS Inc., Chicago, USA) was used. Mean intestinal disaccharidase levels for the dietary groups were compared using one-way analysis of variance followed by Duncan's multiple range test. The disaccharidase activity values obtained when saponins were added in different concentrations, were compared using the *t* test for paired samples. The *t* test for unpaired samples was used to determine whether there was a significant effect of saponins on the rate of breakdown.

RESULTS
Disaccharidase activites in rats fed on different diets

The intake of the guar-gum diet was lower than that of the other diets and consequently the mean final body weight (220 g) was significantly lower in this group. The other dietary

Table 1. *Protein content (mg/g wet tissue) and activities of intestinal disaccharidases (IU/g protein) in the mucosa of rats given different diets**

(Mean values with their standard errors)

Diet	n	Mucosal protein Mean	SE	Maltase (EC 3.2.1.20) Mean	SE	Sucrase (EC 3.2.1.48) Mean	SE	Trehalase (EC 3.2.1.28) Mean	SE	Lactase (EC 3.2.1.21) Mean	SE
Pellets	8	66.0[b]	3.1	299	23	77.9	4.6	107[b]	7	1.3[b]	0.3
Cellulose	8	68.5	2.7	354[a]	13	84.2[a]	5.3	131[a]	4	4.0[a]	0.7
Guar gum	8	82.6[a]	6.6	258[b]	16	68.0	8.1	99[b]	4	3.3[ac]	0.8
EtOH oats†	8	71.2	3.7	293	25	65.0[b]	5.0	105[b]	8	2.0[bc]	0.6
Oats	8	69.0	5.8	302	20	71.1	7.1	111	10	2.8	0.5
EtOH oats + saponins	5	77.1	6.6	294	11	62.5[b]	2.6	112	9	2.4	0.7

[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).
* For details of diets and procedures, see pp. 230–231.
† Ethanol-extracted oats.

groups had similar final body weights ranging from 265 to 282 g. For a more detailed description of weight gain and feed intake, see Önning & Asp (1995).

The protein content and the activities of maltase, sucrase, trehalase and lactase in the intestines of rats fed on different diets are presented in Table 1. The guar gum group had the highest content of mucosal protein, significantly higher than the pellet group. For maltase the activity was highest in the group given cellulose and lowest for the guar gum group. The maltase activities for the groups given oats with different saponin contents were similar. The cellulose group also had the highest activity of sucrase, significantly higher than two of the oat groups, ethanol-extracted oats and ethanol-extracted oats plus saponins. Trehalase activity for the groups fed on oats ranged from 105–112 IU/g protein, the guar gum and pellet groups had similar activities, but again the cellulose group had the highest activity, 131 IU/g protein.

There were larger differences in lactase activities between the groups. The lowest activity was found in the intestines from animals given pellets (1.3 IU/g protein). The activity was similar in the oat groups (2.0–2.8 IU/g protein), and the cellulose group had the highest activity (4.0 IU/g protein). For lactase there was considerable variation in activity within the dietary groups, and the activity was low compared with the other disaccharidases.

Effect of adding saponins on the disaccharidase activity in vitro

The mixture of avenacosides A and B was added in two concentrations (1 and 2 mg/l), and the effects on maltase, sucrase, trehalase and lactase activities are presented in Table 2. There were no significant differences in disaccharidase activities between intestines from the rat groups fed on ethanol-extracted oats and the same diet plus added saponins. Therefore the results from the two groups were pooled (n 6). The maltase activity was significantly ($P < 0.05$) reduced when the concentration of saponins in the incubation medium was 2 mg/ml, but not with the 1 mg/ml concentration, compared with the activity when no saponins were added. There did not seem to be any effect of the saponins on sucrase activity. The activity of trehalase was significantly reduced, by 5% when the concentration of saponins was 1 mg/ml and by 9% at the 2 mg/ml concentration. The lactase activity was also inhibited in the presence of saponins. A reduction of 18% was seen when the highest concentration (2 mg/ml) was used.

Table 2. *Effect of different concentrations of saponins on rat intestinal disaccharidase activities (IU/g protein) in vitro**

(Mean values with their standard errors for six determinations)

Saponin (mg/ml)	Maltase EC 3.2.1.20)		Sucrase (EC 3.2.1.48)		Trehalase (EC 3.2.1.28)		Lactase (EC 3.2.1.21)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	297[a]	12·5	72·1	1·6	110[a]	8·9	2·8	0·7
1	284	10·8	73·5	2·8	104[b]	8·7	2·4[a]	0·5
2	277[b]	8·0	73·3	2·4	100[c]	7·8	2·3[b]	0·5

[a,b,c] Mean values within a column with unlike superscript letters were significantly different, $P < 0.05$.
* For details of procedures, see pp. 230–231.
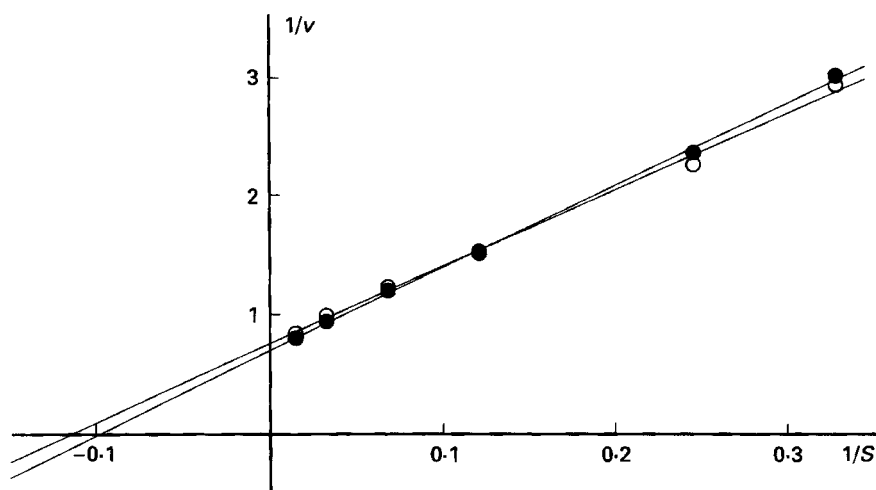
Fig. 1. Effect of oat saponins on trehalase (*EC* 3.2.1.28) activity (Lineweaver–Burk plot). (○), No saponins; (●), 2 mg saponins/ml.

A kinetic study was carried out regarding trehalase and lactase, on which the saponins had a significant inhibiting effect. Lineweaver–Burk plots for trehalase and lactase are presented in Figs. 1 and 2 respectively. A saponin concentration of 2 mg/ml was used. As seen in Fig. 1, the saponins seemed to have no inhibitory effect on the trehalase activity. Thus, the small but statistically significant effect shown in Table 2 was not reproduced. Regarding lactase, reaction kinetics could not be studied with substrate concentrations below 8 mmol/l due to the low activity. At higher substrate concentrations the rate of breakdown was significantly ($P < 0.05$) lower at each point when saponins were present and consequently both V_{max} and K_m were affected. The inhibition was of mixed type; apparent K_m values with and without saponins were 56 and 88 mmol/l respectively, and values for V_{max} were 0·074 and 0·134 IU/ml homogenate solution respectively.

Effect of saponins on α-amylase activity in vitro

As seen in Fig. 3, oat saponins at a concentration of 2 mg/ml increased the hydrolysis of starch significantly compared with the control, especially at the beginning of the time
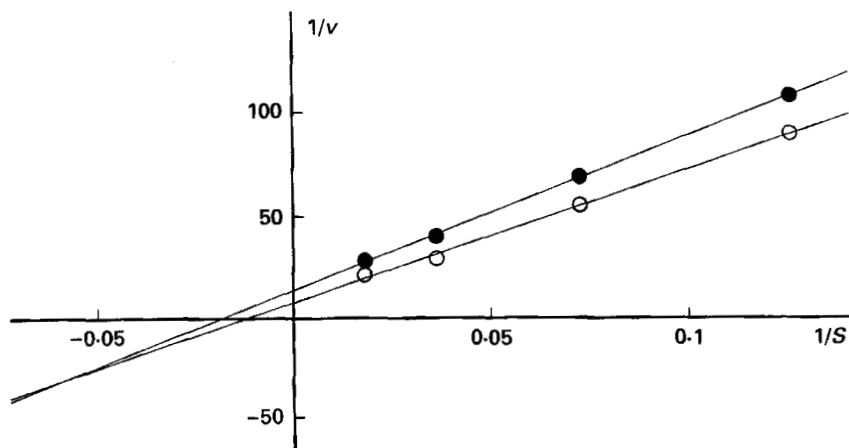
Fig. 2. Effect of oat saponins on lactase (*EC* 3.2.1.21) activity (Lineweaver–Burk plot). (○), No saponins; (●), 2 mg saponins/ml.
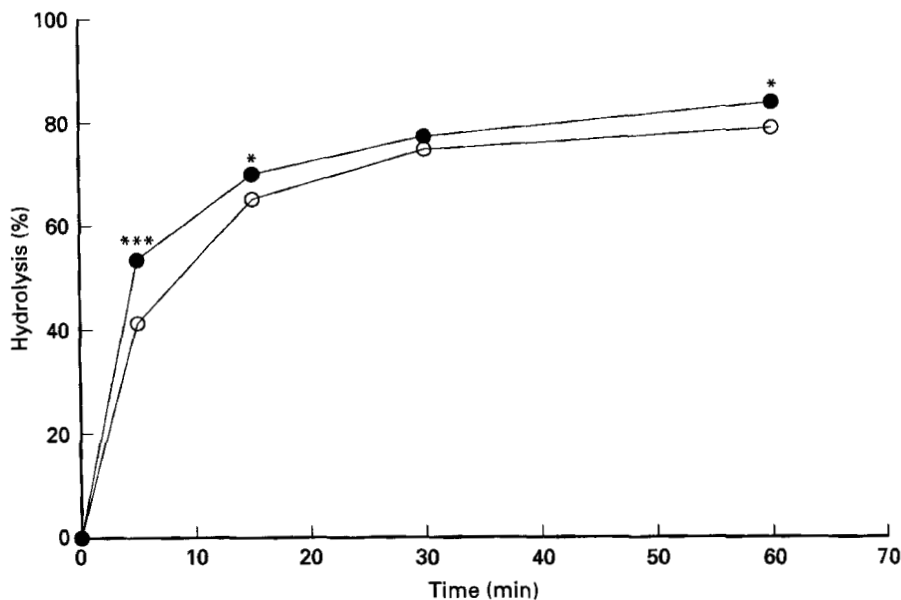

Fig. 3. Effect of oat saponins on starch hydrolysis *in vitro* using a high enzyme concentration (0·2 IU/mg starch). (○), No saponins (*n* 6); (●), 2 mg saponins/ml (*n* 4). The SE did not exceed ±2·0 for any point. Mean values were significantly different from each other: * $P < 0.05$, *** $P < 0.001$. For details of procedures, see p. 231.

interval studied. To study further the early phase of the hydrolysis, the enzyme concentration was decreased 10-fold. The difference in hydrolysis then became more pronounced; in the presence of saponins, 17 % of the starch was hydrolysed after 10 min while in the control only 4 % was degraded (Fig. 4). To find out whether this was a non-specific detergent effect of the oat saponins, Triton X-100 was added to the solutions in the same way as the saponins. Two concentrations (1 and 2 mg/ml) were used. Triton X-100 also increased the rate of starch hydrolysis significantly compared with the control, but not as much as when oat saponins were added (Fig. 4). Thus, there seems to be a relation
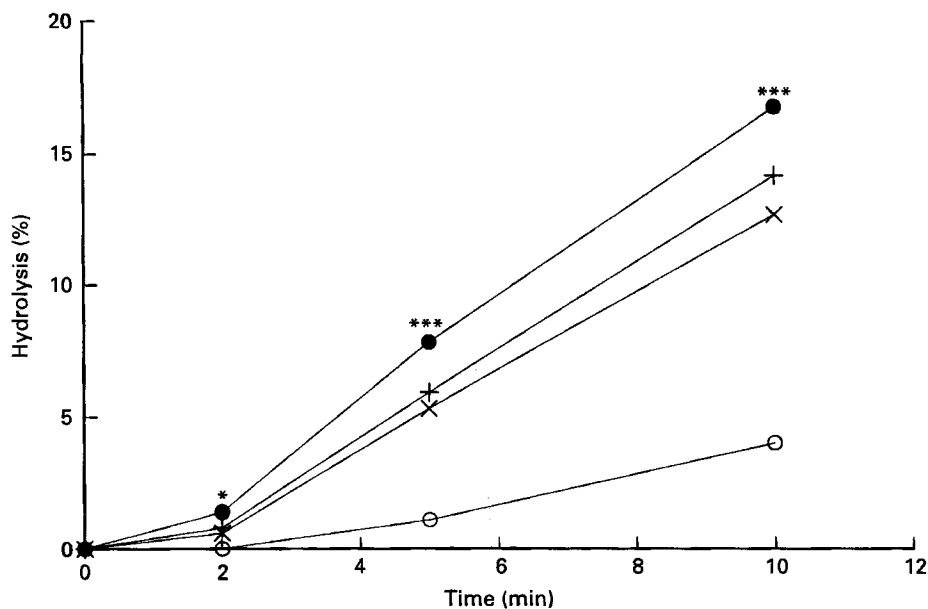
Fig. 4. Effect of oat saponins and Triton X-100 on starch hydrolysis *in vitro* using a low enzyme concentration (0·02 IU/mg starch). (○), No saponins (*n* 6); (●), 2 mg saponins/ml (*n* 4); (×), 1 mg Triton X-100/ml (*n* 4); (+), 2 mg Triton X-100/ml (*n* 4). The SE did not exceed ± 0.9 for any point. Mean values for saponins were significantly different from those for no saponins, * $P < 0.05$, *** $P < 0.001$. For details of procedures, see pp. 231, 234, 235.

between the detergent capability and the degree of starch hydrolysis but other factors could also be involved.

One experiment without preincubation was also made. Starch hydrolysis in the presence of oat saponins was the same as when a preincubation was used. The control values (*n* 4), however, increased when the preincubation was excluded, from 0 to 2·0% at 2 min, from 1·1 to 4·1% at 5 min and from 4·0 to 12·7% at 10 min. Thus, the α-amylase activity was reduced by preincubation for 30 min at 37°, possibly due to aggregation. Nevertheless, the hydrolysis of starch was still significantly higher at 2, 5 and 10 min when saponins were added compared with the control.

DISCUSSION

Feeding rats on oat diets containing different amounts of avenacosides for 19 d did not affect the disaccharidase activities in the proximal part of the small intestine. The concentration of saponins in the diets was quite low (maximum 0·7 g/kg DM) and the concentration in the rat's intestine probably even lower. An indication of the membranolytic activity is the ability of the saponins to lyse erythrocytes. Tschesche & Wiemann (1977) measured the avenacosides' haemolytic activity and found that a concentration of > 1000 μg/ml erythrocyte solution (1 mg/ml) was necessary to get complete haemolysis. The corresponding amount of desglucoavenacosides was much lower (9 μg). Saponin concentrations found in oats are normally quite low. In oat kernels the avenacoside content ranges from 0·20 to 0·50 g/kg DM, depending on the variety (Önning *et al.* 1993*b*). Higher amounts have been found in lucerne (*Medicago sativa*; 17·1 g/kg), soyabeans (6·5 g/kg), kidney beans (*Phaseolus vulgaris*; 3·5 g/kg) and quinoa (*Chenopodium quinoa*; 11·9 g/kg) (Livingston *et al.* 1984; Price *et al.* 1986; Ridout *et al.* 1991).

The different dietary fibres in the diets (soluble and insoluble) seemed to affect the

disaccharidase activities. The diet with the highest amount of soluble fibre (guar gum) gave similar sucrase and lactase activities but a significantly ($P < 0.05$) lower maltase activity than the group that was given a high amount of insoluble fibre (cellulose). Johnson & Gee (1986) gave rats diets containing cellulose or guar gum (100 g/kg) and the rats that were fed on guar gum had, as in the present study, a similar sucrase activity and a lower maltase activity relative to the cellulose group. The lactase activity was also lower in the guar gum group. In a similar study (Johnson *et al*. 1984), all disaccharidase activities (sucrase, maltase and lactase) were decreased by the guar-gum diet, and the length of the small intestine in the guar gum group was increased. In the present study oats gave a lower sucrase activity and similar maltase and lactase activities relative to cellulose. Farness & Schneeman (1982) fed rats on oat bran and compared them with those fed on cellulose; the length of the intestine was the same but, in contrast to the results of the present study, the oats did not affect the sucrase activity.

All groups had low lactase activities (1·3–4·0 IU/g protein) but this is not surprising since adult rats were used. In weanling rats values of over 40 IU/g protein have been found (Thomsen & Tasman-Jones, 1982; Thomsen *et al*. 1983).

The avenacosides inhibited the trehalase and lactase activities *in vitro*. The reduction in activity for trehalase was small, however, and when the substrate concentration was varied no significant reduction was obtained. For lactase the inhibition was more pronounced and it was of mixed type according to the Lineweaver–Burk plot. The V_{max} was lowered in the presence of saponins. One mechanism behind this could be that the saponins combine with the lactase enzyme and in this way reduce the activity. Two β-galactosidases in the rat intestine have been isolated, one with an acid pH optimum and one with a neutral pH optimum (Asp & Dahlqvist, 1968). The neutral β-galactosidase is the main contributor to the lactase activity measured at pH 6.

The oat saponins significantly increased the hydrolysis of starch with α-amylase compared with the control. In a previous study (Ruales & Nair, 1994) quinoa saponins had a tendency to increase the starch hydrolysis with α-amylase. Our experiment with Triton X-100 supports the idea that the mechanism behind this effect is related to the detergent properties of the saponins.

It is difficult to extrapolate results from rat studies to humans but avenacosides in concentrations found normally in oat products probably have no effect on the disaccharidases in the human intestine. Whether, on the other hand, they could affect the intestine in other ways is unclear. Diets containing saponins have been shown to lead to morphological changes in the rat intestine. A diet containing *Gypsophylla* saponins (15 g/kg) gave increased villus height and crypt length in jejunum and ileum, and there were also indications of an enhanced rate of cell proliferation (Gee & Johnson, 1988). Lucerne fed to rats for 4 weeks caused damage to jejunum and colon and both haemorrhagic debris amd ruptures were observed (Story *et al*. 1984). Further research is needed to establish whether oat saponins have significant effects on the intestine.

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