

***Plantago ovata* seeds as dietary fibre supplement: physiological and metabolic effects in rats**

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In rats, the effects of a 4-week supplementation of a fibre-free elemental diet with 100 or 200 g *Plantago ovata* seeds/kg was compared with that of the husks and wheat bran. The seeds increased faecal fresh weight up to 100%, faecal dry weight up to 50% and faecal water content up to 50%. The husks, at the high concentration only, were more effective and wheat bran less effective. Length and weight of the small intestine were not greatly affected by the seeds, but both variables increased significantly in the large intestine. The husks had more pronounced effects, especially in the small intestine, and wheat bran almost no effect. Faecal bacterial mass as estimated from the 2,6-diaminopimelic acid output was increased to the greatest extent by the seed-containing diet and by the high concentration of husks, but to a lesser extent by wheat bran. Faecal and caecal protein content was enhanced by the seeds and wheat bran, but to a lesser extent by the husks. Total acetate in caecal contents or faeces was highest on the seeds and husks diet and not elevated by wheat bran. Total faecal bile acid excretion was stimulated and β -glucuronidase (EC 3.2.1.31) activity reduced by both *Plantago ovata* preparations, but not by wheat bran. Mucosal digestive enzyme activities were inhibited to different degrees by all dietary fibres in the jejunum, and sometimes activated in the ileum. These results suggest that *Plantago ovata* seeds are a partly-fermentable dietary fibre supplement which increases stool bulk; metabolic and mucosa-protective effects are also probable.

***Plantago ovata*: Dietary fibre supplement**

Dietary fibres from natural or semi-synthetic sources have gained increasing attention as a supplement to the normal Western diet, which is poor in bulking substances, and as a prophylactic or therapeutic treatment of large bowel disorders (Dreher, 1987). *Plantago ovata* husks, often referred to as ispaghula (husks) or as psyllium (which is a misleading term as nearly all the commercially-available drug derives from *Plantago ovata* Forsk. and not from *Plantago psyllium* L.) and wheat bran are widely used and well-investigated examples. Both compounds increase stool weight (Stevens *et al.* 1988), but have rather opposite physicochemical properties regarding solubility and water-binding capacity (Eastwood *et al.* 1983; Van Soest, 1984; Englyst *et al.* 1989).

Another fibre source, less well known, is *Plantago ovata* seeds. In contrast to the husks which consist only of the hulls of the seeds, the seed preparation includes the kernel together with the hulls. The water-holding capacity of the seeds is intermediate between that of the husks and wheat bran (Sosulski & Cadden, 1982; Struthers, 1986; Leng-Peschlow, 1990). Clinical studies with a *Plantago ovata* seed preparation indicate that this compound increases stool weight and reduces intestinal transit time in healthy volunteers (Heckers & Zielinsky, 1984) and augments stool frequency, stool mass, and water content in constipated patients (Sölter & Lorenz 1983; Marlett *et al.* 1987). Symptoms in irritable bowel patients are ameliorated and consumption of spasmolytics reduced (Ligny, 1988). There is no information about this fibre derived from animal studies.

Table 1. *Fibre content of the experimental fibre supplements*
(Determined according to the Association of Official Analytical Chemists (Prosky *et al.* 1985))

	TDF (g/kg)	SF:NSF (%)
<i>Plantago ovata</i> : Seeds	860	47:53
Husks	948	67:33
Wheat bran	504	7:93

TDF, total dietary fibre; SF, soluble fibre; NSF, insoluble fibre.

The present experiments in rats were performed to characterize the properties of *Plantago ovata* seeds as a dietary fibre supplement. The husks and wheat bran were used for comparison. A feeding period of 4 weeks was chosen as it has been shown that this time-period is sufficient for most variables to adapt to the diet change (Walter *et al.* 1986).

EXPERIMENTAL

Animals and diets

Seventy female adult Wistar rats supplied by Mus Rattus (Brunnthal, Germany) were caged in pairs under standardized experimental conditions (room temperature $22 \pm 2^\circ$, relative air humidity $43 \pm 2\%$, light-dark cycle 12:12 h). Water and food were freely available. All animals were allocated randomly into seven groups and adapted for 14 d to a low-residue, nutritionally-complete, pelleted elemental diet (ssniff-Spezialitäten, Soest, Germany) containing (g/kg): casein 145, starch 415, sucrose 150, glucose 150, soya-bean oil 20, vitamin mixture 10 (retinol 4 mg, thiamin mononitrate 20 mg, riboflavin 20 mg, pyridoxine hydrochloride 20 mg, cyanocobalamin 30 μ g, L-ascorbic acid 15 mg, cholecalciferol 15 mg, α -tocopherol acetate 140 mg, biotin 150 μ g, menadione 10 mg, calcium-D-pantothenate 60 mg, nicotinamide 50 mg, folic acid 10 mg, myo-inositol 80 mg, p-aminobenzoic acid 80 mg, choline chloride 1,000 mg, rutin 50 mg, orotic acid 60 mg, DL-methionine 1,000 mg) and mineral salt mixture 60 (CaCO₃ 18 g, Ca₅(PO₄)₃(OH) 9.75 g, CoCl₂ 0.5 mg, CuSO₄ · 5 H₂O 25 mg, ferrous fumarate 500 mg, K₂HPO₄ 11 g, KI 0.5 mg, MgSO₄ · 7 H₂O 5.5 g, MnSO₄ · H₂O 450 mg, NaCl 7.6 g, NaF 9.5 mg, Na₂HPO₄ 7.1 g, Na₂MoO₄ · 2 H₂O 0.5 mg, NH₄AL(SO₄)₂ 19 mg, ZnCO₃ 45 mg).

After the adaptation period, finely powdered *Plantago ovata* seeds, including approximately the natural amount of husks, whole *Plantago ovata* husks (both supplied by Madaus AG, Cologne, Germany) or coarse wheat bran (Milupa AG, Friedrichsdorf, Germany) were added to the elemental diet (100 or 200 g/kg). The seventh group (controls) received the elemental diet for the whole experimental period. Each diet was given in a pelleted form to groups of ten rats for 4 weeks. The total fibre content of the supplements was analysed according to the Association of Official Analytical Chemists method (Prosky *et al.* 1985) and the insoluble and soluble components after separation by filtration (Rabe *et al.* 1988). The fibre compositions are given in Table 1 and the particle sizes of each supplement (determined by sieve analysis) in Table 2.

Experimental procedure

Body-weight, food consumption and stool output were measured once weekly. For this purpose, the rats were placed individually in wide-gridded cages for 24 h. Stool collections were made at hourly intervals over 8 h during the day and in a single 16 h period during

Table 2. *Distribution (%) of particle sizes of the dietary fibres*

Particle size (mm)	<i>Plantago ovata</i>		
	Seeds	Husks	Wheat bran
> 1.000	0	29.5	73.3
0.500–1.000	26.9	32.9	16.8
0.250–0.500	58.7	19.6	5.5
0.125–0.250	12.5	5.7	3.6
< 0.125	0.7	0.7	0.3

the night. Fresh and dry weights of the faeces were determined for the daytime collections, dry weight only for the night-time collections. Food consumption was simultaneously measured by reweighing the food residues after 24 h for each animal. The dried faeces of the 2nd and 4th week were frozen at -60° for further analyses (protein, acetate, total bile acids, and 2,6-diaminopimelic acid (DAPA)). β -Glucuronidase (*EC* 3.2.1.31) activity was determined weekly in a freshly collected faecal pellet.

At the end of week 4, in the morning after the last stool collection period, rats were anaesthetized with ketamine + xylazine ((50 + 4) mg/kg body-weight in 4 ml physiological saline (9 g sodium chloride/l) given intraperitoneally) and, after removal of the intestine, killed by an overdose of anaesthetic. The pH of the caecal contents was measured through a small incision *in situ*. The lengths of the small intestine and colon were determined. The whole small intestine, caecum, and colon were weighed immediately with and without contents, the tissues were dried at 60° and reweighed. From the small intestine, three pieces of 100 mm in length (proximal, middle and distal part) were used for tissue weight determinations. Scrapings of the mucosa were immediately taken from two other pieces of 100 mm length (proximal jejunum, distal ileum). The mucosa was homogenized in ice-cold buffer (Farness & Schneeman, 1982) and frozen at -60° until enzyme determinations.

A sample of fresh caecal contents was preserved in an ice bath for subsequent analysis of β -glucuronidase, the rest was dried and frozen at -60° for further determinations.

Analyses

β -Glucuronidase in faeces and caecal contents was determined according to Shiao & Chang (1983), DAPA according to Czerkawski (1974), acetate by a commercially-available enzymic test kit (Boehringer Mannheim, Mannheim, Germany), total 3α -hydroxy bile acids after extraction from faeces (Evrard & Janssen, 1968) using an enzymic test kit (Merck, Darmstadt, Germany) and protein, after homogenization and extraction from faeces or caecal contents with 0.1 M-sodium hydroxide (3 h, 50°), in the supernatant fraction using a protein assay kit (Sigma, Deisenhofen, Germany).

Small intestinal mucosal enzymes (alkaline phosphatase *EC* 3.1.3.1. (AP), sucrase *EC* 3.2.1.48, leucine aminopeptidase *EC* 3.4.11.1 (LAP), α -amylase *EC* 3.2.1.1) and mucosal protein were determined in the thawed homogenates using commercially-available test kits for protein (Sigma, Deisenhofen, Germany), AP (Hoffmann La Roche, Grenzach, Germany), LAP and α -amylase (Boehringer, Mannheim, Germany). Sucrase was analysed according to Dahlqvist (1970) using a glucose test kit (Boehringer, Mannheim, Germany).

All results are given as means and standard deviations. Fibre-supplemented groups are compared with the fibre-free control by Student's *t* test using a two-tailed evaluation. In the case of unequal variances, the Welch test was used.

RESULTS

Body-weight and food intake

Food intake after the sudden change to the fibre-enriched diet was reduced to the greatest extent in the groups receiving the diet with *Plantago ovata* husks, to a lesser extent in the seed-diet groups and still less in the wheat-bran-diet groups compared with the fibre-free controls. After 4 weeks there were no significant differences compared with the control group, but there was a tendency to an increased consumption of the high-fibre-content diets compared with the corresponding diet with half the fibre content (Table 3).

Body-weight gain with the seed- or husk-containing diets was comparable with that of the fibre-free control diet. Both wheat-bran groups, but most markedly the high-fibre group, showed a smaller gain. The 200 g wheat bran/kg group, however, had initially higher body-weights in spite of the randomization procedure. As food intake was similar to that of the other groups, the food conversion efficiency was lower in both wheat-bran groups (Table 3).

Faecal excretion

The faecal pellets of the fibre-free group were small and hard. *Plantago ovata* seeds and husks increased the size of the pellets and their number by up to 100%. Although the pellets were well-formed in both groups, their consistency was soft and the interior, especially with the husk-diet group, was like gum. Wheat bran increased the number of faecal pellets by 20–50%, but did not change their consistency.

There were some variations in faecal excretion during the 4 weeks but, principally, similar results were obtained for the same diet after 1 week or 4 weeks of treatment. Therefore, the results from all four sampling occasions for each dietary group were combined (Table 3).

Daily faecal dry weight significantly increased only with the 200 g husks/kg diet, but there was the same tendency for the other fibre-rich diets compared with the fibre-free controls. Water content and fresh weight of the faeces was pronouncedly and dose-dependently higher with both *Plantago ovata* diets but not with wheat bran. Daily fresh weights represent a rough estimation since they are based on the mean faecal water content of the day's collection periods, not available from all animals.

Intestinal lengths and weights

The length of the small intestine increased with the seed-containing diet by 4–8% and with the husk-containing diet by 13–24% (Table 4). Wheat bran did not influence small intestinal length. In the colon, both *Plantago ovata* preparations induced a 10–20% increase in length, bran had an effect only in the high dose (10% increase).

The total fresh weight of the small intestine was 30–40% higher in rats fed on seeds and 85–150% higher in the husk-fed groups, whereas there was little or no change with wheat bran. *Plantago ovata* seeds increased caecal weight by 116–145%, husks by 141–286% and bran by 13–29%. The corresponding values for total colon weight were 47–110% for seeds, 90–202% for husks and 0–42% for wheat bran.

The amount of small intestinal contents estimated by total weight, total length and average tissue weight based on three segments was negligible in the fibre-free and low-wheat-bran groups and was less than 10% of the total fresh weight of the small intestine in the seed-fed and high-wheat-bran groups. The contents represented, however, approximately 30% of the total small intestinal wet weight in both husk-fed groups. Caecal contents accounted for 72% of the total caecal wet weight in the fibre-free group, 85% in all *Plantago ovata* preparations and 77% in the wheat-bran groups. In the colon, the contents contributed 50% of the total fresh weight in fibre-deficient rats, 61–69% in the

Table 3. The effect of dietary fibre supplements (Plantago ovata seeds or husks or wheat bran at 100 and 200 g/kg) on body-weights, food consumption, and faecal excretion of rats during a 4-week period
(Mean values and standard deviations for ten rats/group)

Diet ...	Fibre-free		Plantago ovata seeds		Plantago ovata husks		Wheat bran	
	Mean	SD	100	200	100	200	100	200
Fibre level (g/kg) ...								
Body-wt:								
Initial (g)	211	10	222*	216	11	218	12	215
Final (g)	234	15	246	236	16	242	18	230
Gain (%)	10.5	5.8	10.5	9.4	2.8	10.9	5.7	7.3
Food intake (g/kg body-wt per 24 h):								
Initial	60.4	7.8	52.3*	53.8	9.5	49.3**	6.9	58.3
Final	61.1	10.6	59.4†	62.4	11.6	57.3†	5.8	59.2
Food conversion efficiency (%)‡	5.9	3.3	6.1	5.6	1.4	6.1	2.9	4.3
Faeces (g/kg body-wt per 24 h):								
Dry wt§	5.5	3.1	6.4	7.6***	2.1	11.3***	2.9	7.9***
Wet wt	11.2	6.3	16.2***	22.3***	7.7	38.9***	11.4	16.3***
Water content (g/kg faeces)¶	480	120	600*	650**	50	710	30	514
Mean								
SD								
Mean								
SD								

Mean values were significantly different from those of the fibre-free group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Final intakes were significantly different from the initial intakes for the same dietary group: † $P < 0.05$.

‡ Food conversion efficiency calculated from body-weight gain \times 100/food intake (g).

§ Averaged over four 24 h periods at weekly intervals (n 40).

|| Estimated from dry weight/24 h and mean water content of the fresh daily samples of each rat. Where no individual values were available, mean group water content for each period was used for calculation.

¶ Averaged over all sampling occasions for each individual animal (n 4-8, as not all rats excreted faeces during the day).

Table 4. The effect of dietary fibre supplements (Plantago ovata seeds or husks or wheat bran at 100 or 200 g/kg) on intestinal lengths and weights of rats during a 4-week period
(Mean values and standard deviations for ten rats/group)

Diet ...	Fibre-free		Plantago ovata seeds		Plantago ovata husks		Wheat bran								
	Mean	SD	100	200	100	200	100	200							
Fibre level (g/kg) ...															
Length (mm):															
Small intestine	1040	50	1080	90	1120*	70	1170***	80	1290***	120	1010	50	1020	70	
Colon	126	10	138*	11	152**	22	140*	12	153***	5	124	8	136	16	
Total fresh wt (g):															
Small intestine	5.5	0.6	7.4***	1.1	7.7***	1.3	10.2***	1.9	13.6***	2.5	5.0	0.7	6.5*	1.2	
Caecum	2.7	0.3	5.7***	0.8	6.5***	1.3	6.4***	1.5	10.3***	2.5	3.0	0.7	3.4*	0.8	
Colon	2.3	0.6	3.4**	0.7	4.8***	1.4	4.4***	1.2	6.9***	1.6	1.9	0.3	3.3**	0.8	
Contents wet wt (g):															
Small intestine†	-0.75	0.32	0.60***	0.69	0.78***	0.92	2.87***	1.22	4.59***	1.64	-0.23***	0.26	0.51***	0.66	
Caecum	1.95	0.36	4.84***	0.74	5.56***	1.21	5.46***	1.28	8.91***	2.22	2.31	0.72	2.61*	0.76	
Colon	1.14	0.58	2.07**	0.55	3.30***	1.29	2.94***	1.08	5.04***	1.42	0.88	0.33	2.04**	0.73	
Contents dry wt (g)‡:															
Caecum	0.32	0.07	0.58***	0.07	0.63***	0.12	0.48***	0.19	0.75***	0.16	0.46*	0.14	0.60**	0.19	
Water content (g/kg)‡:															
Caecum contents	834	12	880***	12	885***	10	910***	12	915***	10	801***	20	772***	17	

Mean values were significantly different from those of the fibre-free group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Estimated from total fresh weight and total tissue wet weight (calculated from small intestinal length and mean duodenal, jejunal, and ileal tissue weight/100 mm).

‡ Not determined for small intestine and colon.

seed-containing diets, 67–73% in the husk-containing diets, and 46–62% in the wheat-bran diets. Water content of food residues, which was only measured in the caecum, was 83% in fibre-free rats and even less in the wheat-bran groups, and increased to 88 and 91% for diets containing *Plantago ovata* seeds and husks respectively.

Tissue weight (Table 5) per 100 mm length was not influenced in the upper small intestine by any of the dietary fibres but increased in the middle and lower parts of the small intestine and in the colon in the presence of the husks, especially at the high concentration. *Plantago ovata* seeds, 100 and 200 g/kg, and wheat bran, 200 g/kg, had a significant effect in the caecum only which was, however, moderate compared with that with the husks. Tissue water content in the caecum and colon rose highly significantly in both husk-fed groups and less in the 200 g seeds/kg and wheat-bran groups.

Bacteria-dependent variables in caecal contents and faeces

As dietary fibres individually affect the intestinal contents in various respects (water binding, non-degradable residue, bacterial mass), dilution problems complicate comparisons between the different groups based on concentration values related to g wet weight, g dry weight, protein content etc. Therefore, absolute amounts, related to 24 h faecal output or whole caecal contents, are given in addition to concentrations expressed on a dry weight basis (Table 6). As an exception, the activity of β -glucuronidase, which was determined in a single fresh faecal pellet, was related to wet weight only. Standard deviations of the calculated absolute amounts in faeces were rather high, as total faecal excretion sometimes varied considerably within a group.

DAPA. The concentration in faeces significantly increased in the *Plantago ovata* seed diet compared with the fibre-free treatment, both the other fibre types had no effect. The total amount of DAPA in a 24 h faeces sample was doubled in the two seed-fed groups and in the 200 g husks/kg group. Wheat bran showed only a trend towards a higher amount.

Protein. The concentration in faeces was similar to that of the fibre-free control in the seed-fed and wheat-bran-fed rats, but decreased in both husk-fed groups. A significant dilution by an increased faecal dry weight mass was obvious in the 200 g husk/kg group and less so in the 200 g seeds/kg group and both wheat-bran groups, but not in the 100 g husks or seeds/kg groups compared with the fibre-free control. In caecal contents, protein concentration was reduced by all fibre-rich diets except 200 g wheat bran/kg. There was no direct correlation between protein concentration and dilution by an increased caecal dry weight mass in the different groups, as the same dry weight increase coincided with a decrease in protein concentration in the 200 g seeds/kg group and an increase in the 200 g wheat bran/kg group. Total protein content in faeces clearly increased with *Plantago ovata* seeds and wheat bran and was affected in the husk-fed group only at the 200 g/kg concentration. In total caecal contents, the results were the same, but the husks, in a high concentration, increased protein content also.

β -Glucuronidase. The activity, related to g wet weight, showed a time-dependent decrease over 4 weeks in the faeces of rats receiving 100 or 200 g *Plantago ovata* seeds/kg and 100 g husks/kg, finally reaching 25% of the activity of the control group. Faecal water content did not change considerably within the groups during this time. The high-husk dietary group showed a sudden and pronounced reduction within the first 8 d, falling to 10% of the activity of the fibre-free group, whereas wheat bran at a concentration of 100 g/kg had no effect and at 200 g/kg led to a 50% reduction. In caecal contents, β -glucuronidase activity decreased in both *Plantago ovata* preparations but not in the wheat bran group which may be partly explained by the changes in water content. Although dry weight had not been determined in the faecal pellets used for β -glucuronidase determinations, an attempt was made to calculate total faecal β -glucuronidase output on the basis of the 24 h dry matter and the mean water content of the fresh faeces of each rat (or, if not available,

Table 5. The effect of dietary fibre supplements (Plantago ovata seeds or husks or wheat bran at 100 or 200 g/kg) on intestinal tissue weights of rats during a 4-week period

(Mean values and standard deviations for ten rats/group)

Diet...	Fibre-free		Plantago ovata seeds		Plantago ovata husks		Wheat bran	
	Mean (SD)	100 Mean (SD)	200 Mean (SD)	100 Mean (SD)	200 Mean (SD)	100 Mean (SD)	200 Mean (SD)	
Fibre level (g/kg) ...								
Tissue wet wt (g)								
Duodenum (100 mm)	0.83 (0.10)	0.84 (0.09)	0.83 (0.07)	0.77 (0.08)	0.77 (0.06)	0.73 (0.08)*	0.83 (0.07)	
Jejunum (100 mm)	0.52 (0.09)	0.56 (0.08)	0.53 (0.04)	0.60 (0.06)*	0.66 (0.13)*	0.46 (0.06)	0.50 (0.06)	
Ileum (100 mm)	0.45 (0.07)	0.48 (0.10)	0.51 (0.09)	0.50 (0.05)	0.68 (0.11)***	0.38 (0.04)*	0.43 (0.07)	
Caecum (total)	0.71 (0.06)	0.91 (0.10)***	0.97 (0.08)***	0.94 (0.25)*	1.36 (0.33)***	0.70 (0.05)	0.80 (0.08)**	
Colon (100 mm)	0.93 (0.14)	0.96 (0.10)	1.01 (0.12)	1.02 (0.14)	1.25 (0.22)***	0.86 (0.09)	0.90 (0.11)	
Tissue dry wt (g)								
Duodenum (100 mm)	0.20 (0.03)	0.20 (0.03)	0.19 (0.02)	0.18 (0.02)	0.18 (0.02)	0.17 (0.02)	0.20 (0.02)	
Jejunum (100 cm)	0.13 (0.04)	0.13 (0.03)	0.12 (0.02)	0.12 (0.01)	0.14 (0.03)	0.12 (0.03)	0.12 (0.03)	
Ileum (100 mm)	0.10 (0.03)	0.11 (0.04)	0.11 (0.03)	0.11 (0.01)	0.15 (0.03)***	0.08 (0.01)	0.09 (0.03)	
Caecum (total)	0.19 (0.03)	0.24 (0.03)**	0.23 (0.03)*	0.21 (0.04)	0.27 (0.04)***	0.17 (0.02)	0.19 (0.02)	
Colon (100 mm)	0.29 (0.05)	0.27 (0.05)	0.27 (0.06)	0.25 (0.03)*	0.28 (0.05)	0.26 (0.03)	0.26 (0.03)	
Tissue water content (g/kg)								
Duodenum	764 (25)	760 (11)	769 (14)	773 (6)	770 (9)	763 (13)	755 (7)	
Jejunum	758 (41)	774 (39)	780 (35)	797 (20)*	787 (22)	751 (38)	762 (43)	
Ileum	784 (37)	764 (55)	787 (41)	771 (23)	784 (21)	785 (23)	783 (31)	
Caecum	732 (33)	738 (23)	766 (18)*	772 (14)**	799 (26)***	757 (22)	762 (30)*	
Colon	687 (27)	716 (24)*	731 (26)**	757 (7)***	772 (9)***	700 (36)	716 (22)*	

Mean values were significantly different from those of the fibre-free group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 6. The effect of dietary fibre supplements (Plantago ovata seeds or husks or wheat bran at 100 and 200 g/kg) on intestinal-bacteria-dependent variables determined in faeces and caecal contents of rats during a 4-week period

(Mean values and standard deviations for seven to ten rats/group. Concentrations expressed per g dry faeces or caecal contents, absolute amounts related to faeces collected over 24 h or to the total caecal contents)

Diet ...	Fibre-free		Plantago ovata seeds		Plantago ovata husks		Wheat bran		
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Fibre level (g/kg) ...									
		100	200	100	200	100	200	100	200
DAPA									
Faeces: day 29	0.66 (0.13)	0.91 (0.06)***	1.03 (0.15)***	0.72 (0.20)	0.73 (0.15)	0.70 (0.11)	0.60 (0.16)	0.70 (0.11)	0.60 (0.16)
mg/g dry wt	0.79 (0.58)	1.51 (0.59)*	1.70 (0.61)**	0.85 (0.32)	1.87 (0.53)***	1.29 (0.65)	1.12 (0.33)	1.29 (0.65)	1.12 (0.33)
mg/24 h									
Protein: (mg/g dry wt)									
Faeces: Day 15	127 (18)	147 (12)**	143 (14)*	105 (9)**	74 (10)***	122 (11)	137 (11)	122 (11)	137 (11)
Day 29	142 (14)	140 (10)	155 (15)	95 (9)***	80 (12)***	133 (15)	137 (15)	133 (15)	137 (15)
Caecal contents	159 (11)	130 (11)***	134 (15)***	113 (11)***	107 (14)***	144 (14)*	180 (15)**	144 (14)*	180 (15)**
(mg/24 h or caecum)									
Faeces: Day 15	143 (65)	210 (51)*	245 (35)**	132 (37)	204 (45)*	210 (63)*	231 (95)*	210 (63)*	231 (95)*
Day 29	184 (154)	229 (82)	251 (69)	119 (49)	200 (44)	231 (85)	261 (79)	231 (85)	261 (79)
Caecal contents	52 (12)	75 (12)***	84 (17)***	55 (13)	81 (26)**	65 (17)	108 (35)***	65 (17)	108 (35)***
β -Glucuronidase (EC 3.2.1.31):									
(μ mol/g wet wt)†									
Faeces: Day 8	7.41 (2.93)	8.36 (4.55)	8.76 (2.15)	8.13 (4.35)	1.79 (1.34)***	7.33 (5.11)	3.97 (2.79)*	7.33 (5.11)	3.97 (2.79)*
Day 15	6.19 (3.24)	6.61 (4.24)	6.66 (3.92)	4.08 (2.69)	0.39 (0.48)**	5.75 (4.74)	4.50 (3.61)	5.75 (4.74)	4.50 (3.61)
Day 22	6.65 (3.63)	4.26 (3.09)	2.84 (1.33)*	3.66 (0.91)*	0.56 (0.33)**	6.29 (5.39)	3.50 (1.84)*	6.29 (5.39)	3.50 (1.84)*
Day 29	10.75 (5.08)	2.44 (1.60)**	2.70 (2.19)***	2.85 (2.53)**	0.97 (0.66)***	8.87 (9.77)	5.17 (3.05)**	8.87 (9.77)	5.17 (3.05)**
Caecal contents	2.32 (1.36)	0.62 (0.40)**	0.63 (0.36)	0.27 (0.26)**	0.31 (0.26)**	4.71 (3.48)	3.49 (1.59)	4.71 (3.48)	3.49 (1.59)
(μ mol/24 h or caecum)									
Faeces: Day 8	15.0 (6.6)	32.6 (16.2)*	52.8 (18.9)***	—	12.7 (9.4)	15.9 (7.8)	11.6 (9.1)	15.9 (7.8)	11.6 (9.1)
Day 15	13.2 (9.7)	21.9 (9.7)	32.9 (18.2)*	12.1 (7.5)	2.9 (3.4)*	16.6 (9.5)	14.4 (12.6)	16.6 (9.5)	14.4 (12.6)
Day 22	16.7 (13.0)	12.4 (6.6)	13.0 (6.7)	12.9 (6.2)	5.2 (2.8)*	20.7 (16.8)	18.9 (13.7)	20.7 (16.8)	18.9 (13.7)
Day 29	22.0 (14.6)	8.7 (4.8)*	11.0 (8.4)	—	8.2 (5.1)*	15.1 (9.8)	20.2 (17.4)	15.1 (9.8)	20.2 (17.4)
Caecal contents	4.7 (3.3)	3.0 (1.9)	3.6 (2.5)	1.4 (1.2)*	2.7 (2.2)	6.6 (2.1)	8.2 (3.1)*	6.6 (2.1)	8.2 (3.1)*

DAPA, diaminoipimeic acid.

Mean values were significantly different from those of the fibre-free group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† μ mol phenolphthalein released during 20 min incubation; dry weight not determined.

of the group) collected on the preceding day. An initial increase in total faecal β -glucuronidase activity was found for the seeds (indicating that the effect on enzyme inhibition was delayed compared with the increase in stool weight) followed by a decrease until the end of the experimental period. In parallel with the concentration findings, the decrease was more rapid in the high-husk dietary group. Wheat bran induced no change compared with the fibre-free control. In total caecal contents, differences in the β -glucuronidase activity between the groups were not as pronounced as in faeces except for a low activity in the 100 g husk/kg group and a high activity in the 200 g wheat bran/kg group.

Fermentation and short-chain fatty acids (SCFA)

Total amounts of acetate as a main product of bacterial fermentation showed a pronounced increase in caecal contents and faeces of rats fed with *Plantago ovata* seeds or husks but generally not in wheat-bran-fed rats (Table 7). There were no important differences between the two *Plantago ovata* preparations. The pH of the caecal contents did not correlate with acetate in that an increasing concentration of SCFA lowered pH. Wheat-bran-fed rats with the lowest acetate concentration had the most acid pH in the caecum.

Total bile acids

The concentration of the 3α -hydroxy bile acids and their total amounts significantly increased in dried caecal contents and faeces of rats fed on either of the two *Plantago ovata* preparations (Table 7). The effect was more pronounced in the husk-fed group than in the seed-fed group. There was little change in wheat-bran-fed animals compared with the fibre-free group. On a wet weight basis (values not shown), the bile acid concentration in caecal contents did not significantly differ from that of the fibre-free controls in all treated groups with the exception of the 200 g seeds/kg group which was elevated by 56%.

Mucosal enzymes in the small intestine

Plantago ovata seeds produced a dose-dependent reduction in the activity of AP in the jejunum by 31 and 92%, of sucrase by 16 and 30%, of LAP by 3 and 53%, and of α -amylase by 53 and 26% respectively (Table 8). The other two dietary fibres depressed AP activity, already at a low concentration, by more than 90%, sucrase activity by 7–21%, LAP activity by 47–58% and amylase activity by 39–57%.

In the ileum of the control rats, all enzyme activities were lower than in the jejunum, especially AP, sucrase, and α -amylase. Changes in enzyme activities due to the fibre-enriched diet were less dramatic than in the upper region. In contrast to the jejunum, AP activity was significantly increased 2–3-fold by the two *Plantago ovata* preparations and, to a lesser extent, by wheat bran. Sucrase activity was almost unaffected. LAP and α -amylase activities were reduced by the diets containing seeds and husks and only α -amylase was reduced, by 20%, with wheat bran.

DISCUSSION

Faecal bulking

The effect of a dietary fibre on faecal bulking is mainly due to the presence of its indigestible residue, the water-binding capacity of this residue and on the bacterial mass which depends on the amount of fermentable substrate in the large intestine (Cummings, 1984). *Plantago ovata* husks, as a highly-soluble fibre, are digested to 70–90% by the large intestinal microflora (Prynne & Southgate, 1979; Nyman & Asp, 1985). Fibres with a high content of insoluble carbohydrates are more resistant. Wheat bran is broken down only to about 40% (Nyman & Asp, 1982; Nyman *et al.* 1986). The degree of bacterial degradation of *Plantago ovata* seeds is not known, but based on its fibre composition it may be supposed

Table 7. The effect of dietary fibre supplements (Plantago ovata seeds or husks or wheat bran at 100 or 200 g/kg) on acetate and 3 α -hydroxy bile acids in faeces and caecal contents of rats during a 4-week period
(Mean values and standard deviations for nine to ten rats/group).

Diet...	Fibre-free		Plantago ovata seeds		Plantago ovata husks		Wheat bran	
	Mean (SD)	Mean (SD)	100	200	100	200	100	200
Fibre level (g/kg)...			Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Acetate: (μ mol/g dry wt)								
Faeces: Day 15	61 (23)	109 (47)*	146 (40)***	123 (54)**	110 (19)***	86 (27)*	74 (26)	
Day 29	56 (16)	114 (34)***	121 (36)***	96 (33)**	129 (50)**	66 (28)	45 (17)	
Caecal contents	301 (202)	525 (90)**	516 (80)*	707 (115)***	553 (156)**	288 (65)	202 (58)	
(μ mol/24 h or caecum)								
Faeces: Day 15	70 (43)	165 (100)*	262 (114)***	174 (133)*	310 (108)***	153 (71)**	135 (83)	
Day 29	86 (95)	195 (106)*	212 (115)*	132 (82)	355 (196)**	136 (103)	86 (46)	
Caecal contents	99 (67)	301 (49)***	325 (77)***	339 (82)***	402 (107)***	140 (59)	126 (54)	
pH								
Caecal contents	7.11 (0.34)	6.93 (0.15)	6.81 (0.16)*	7.20 (0.12)	6.97 (0.20)	6.78 (0.22)*	6.10 (0.24)***	
Bile acids: (μ mol/g dry wt)								
Faeces: Day 15	1.53 (0.26)	3.68 (0.78)***	4.01 (1.39)***	5.65 (1.46)***	5.14 (1.74)***	1.76 (0.77)	1.80 (0.37)	
Day 29	2.15 (0.43)	2.88 (0.51)**	4.01 (1.38)**	5.04 (1.29)***	4.75 (1.01)***	1.28 (0.38)***	1.40 (0.47)**	
Caecal contents	2.24 (0.82)	3.00 (0.93)	5.09 (1.23)***	4.60 (1.92)**	4.18 (2.10)*	1.95 (0.41)	1.61 (0.37)	
(μ mol/24 h or caecum)								
Faeces: Day 15	1.71 (0.82)	5.26 (1.63)***	7.29 (3.80)**	7.28 (3.26)***	14.01 (5.22)***	2.94 (1.16)*	3.01 (1.26)*	
Day 29	3.10 (3.14)	4.54 (1.52)	6.60 (3.28)*	5.94 (2.18)*	11.93 (1.91)***	2.35 (1.22)	2.74 (1.42)	
Caecal contents	0.70 (0.22)	1.70 (0.45)***	3.22 (1.00)***	2.21 (0.99)**	2.94 (1.22)***	0.90 (0.41)	1.08 (0.33)**	

Mean values were significantly different from those of the fibre-free group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 8. The effect of dietary fibre supplement (Plantago ovata seeds or husks or wheat bran at 100 or 200 g/kg) on mucosal enzymes in the small intestine of rats after a 4-week period

(Mean values and standard deviations for ten rats/group. Values expressed as mU/mg protein).

Diet ...	Fibre-free		Plantago ovata seeds		Plantago ovata husks		Wheat bran	
	Mean (SD)	100 Mean (SD)	200 Mean (SD)	100 Mean (SD)	200 Mean (SD)	100 Mean (SD)	200 Mean (SD)	
Fibre level (g/kg) ...								
Jejunum:								
AP (EC 3.1.3.1)	187.3 (33.0)	132.9 (46.4)**	15.8 (4.5)***	7.1 (4.1)***	10.5 (16.1)***	9.0 (6.5)***	6.1 (1.7)***	
Sucrase (EC 3.2.1.48)	102.4 (10.7)	86.5 (12.4)**	72.0 (12.2)***	80.8 (9.9)***	83.0 (9.2)***	81.1 (16.1)**	95.3 (6.6)	
LAP (EC 3.4.11.1)	13.7 (1.7)	13.3 (1.8)	6.5 (1.1)***	6.2 (1.2)***	7.2 (1.5)***	6.1 (0.5)***	5.7 (1.2)***	
α -Amylase (EC 3.2.1.1)	65.7 (41.0)	30.8 (11.7)*	48.3 (39.3)	34.3 (22.1)	29.3 (12.3)*	39.8 (21.1)	28.5 (11.6)*	
Ileum:								
AP	3.8 (1.9)	10.6 (3.5)***	11.1 (6.0)**	12.9 (6.0)**	13.4 (3.5)***	6.5 (1.9)**	6.0 (1.5)**	
Sucrase	23.5 (5.3)	22.8 (6.8)	21.1 (5.7)	27.0 (4.2)	22.8 (5.7)	20.4 (3.2)	16.0 (4.0)**	
LAP	10.9 (3.8)	7.6 (1.6)*	7.7 (1.4)*	5.5 (1.5)**	6.5 (2.7)**	9.0 (1.2)	8.9 (1.4)	
α -Amylase	26.9 (18.5)	9.0 (4.9)*	10.9 (7.1)*	8.2 (3.4)*	9.0 (5.1)*	16.4 (7.6)	8.3 (2.8)*	

AP, alkaline phosphatase; LAP, leucine aminopeptidase.

Mean values were significantly different from those of the fibre-free group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

that its digestibility should be less than that of the husks and greater than that of wheat bran. The particle size of fibre has also been shown to be important for digestibility and stool weight (Brodrribb & Groves, 1978). Fine bran has a lower water-holding capacity, probably a higher digestibility and a weaker effect on stool weight than coarse bran. A comparison of the sieve analysis values from the present study with those of Brodrribb & Groves (1978) show that the bran used in the present study was of a coarse nature. The predominating particle size of *Plantago ovata* husks was smaller than wheat bran, and that of the seeds even smaller, but still above 0.5 mm.

The increase in faecal wet weight in the 100 g fibre/kg diets was similar for all fibres, but in the 200 g fibre/kg diets pronounced differences were seen, following the order husks > seeds > wheat bran. Dose-dependencies were evident for both forms of *Plantago* fibre, but not for wheat bran. The increased faecal water content was mainly associated with the bulking effect of the husks, and also with that of the seeds, whereas in wheat-bran-fed rats faecal water content was in the range of the fibre-free controls. These findings relate to the water-holding capacity which is very high for husks, moderate for seeds and low for wheat bran (Leng-Peschlow 1990).

In relation to the increase in faecal dry weight, wheat bran was more effective than *Plantago ovata* seeds and husks except for the high-husk diet. On the basis of the predicted level of indigestible fibre residues, the effect of the high-husk diet is difficult to explain. A particularly high contribution of the biomass to faecal dry weight due to extensive fermentation and growth of bacteria in the large intestine is not indicated by the measured bacterial markers (DAPA, protein) in the 200 g *Plantago ovata* husk/kg group. Thus, it might be speculated that due to the large volume of the intestinal contents induced by the high-husk diet, transit of digesta in the colon is mostly accelerated in this group and, therefore, a greater proportion of fibre escaped digestion. It is well known that colon motility and propulsion of contents is influenced by the load of material (Bardon & Fioramonti, 1983; Read, 1986) and that the retention time determines the degree of digestion of fibre components (Sakaguchi *et al.* 1987).

2,6-Diaminopimelic acid

This is a constituent of the bacterial cell wall and can be regarded as a marker of bacterial mass (Work & Dewey, 1953; Hutton *et al.* 1971). With a standard diet, bacterial mass amounts to approximately 50% of dry faecal matter in humans (Stephen & Cummings, 1980). In the present study, total faecal DAPA excretion and DAPA concentration in the faeces was highest with the diet containing *Plantago ovata* seeds and not significantly different from that of the fibre-free control with the wheat-bran diet. The latter confirms results of Walter *et al.* (1988). As increased fermentation favours the growth of bacterial mass, these results indicate a higher digestibility of the seeds compared with wheat bran. A high DAPA excretion was also expected for the diet containing husks, but this was only evident in the 200 g/kg group due to the high output of faecal material and not because of an increase in DAPA concentration.

Faecal protein excretion

This may also be used as a marker of bacterial mass, although part of the protein may be of non-bacterial origin, e.g. sloughed intestinal cells or nutritional protein which escaped digestion. Daily faecal protein excretion was highest with the diets containing wheat bran and *Plantago ovata* seeds, while the fibre-free control and the husk-containing diet were similar to each other, which is partly in contrast to the DAPA results. In caecal contents, total protein was also highest with the wheat-bran diet, whereas there was a similar amount in both *Plantago ovata* preparations. Wheat bran contains significant amounts of protein (Nyman & Asp, 1982) and there might be a good chance that protein which is included in

a poorly-digestible fibre matrix is largely protected from enzymic degradation and absorption. *Plantago ovata* seeds also contain some protein, but husks are nearly protein-free (D. A. T. Southgate, personal communication; J. B. Robertson and P. J. Van Soest, personal communication). As the particle size and, thereby the preserved fibre structure, plays a role in the properties of a dietary fibre and also in the accessibility to the fibre constituents (Mongeau & Brassard, 1982), it is likely that protein from the finely-powdered *Plantago ovata* seeds may be more easily available than that from the coarse wheat bran. Thus, undegraded protein may contribute more to the caecal and faecal protein contents with the wheat-bran diet than with the diet containing *Plantago ovata* seeds and plays no role with the husk-containing diet.

Short-chain fatty acids

A third indicator of bacterial activity is SCFA. SCFA are the predominant products of bacterial fermentation in the large intestine and acetate comprises 50–60% of the total amount, the rest being mainly propionate and butyrate (Cummings & Branch, 1986). In the present study, the SCFA analysis has been limited to acetate due to methodological reasons. The amount of acetate in the intestinal contents is the net result of microbial production and its disappearance by absorption. SCFA are rapidly absorbed from the hind-gut (Leng-Peschlow & Marty, 1979; Rechkemmer *et al.* 1988). The absence of an increase in lumen SCFA does not necessarily imply no increase in production, as this is a question of the absorption rate and capacity. The levels of SCFA decrease along the large intestine (Mitchell *et al.* 1985) and it has been found that stool SCFA concentrations remain rather stable despite a variety of dietary or pathological conditions (McKay & Eastwood, 1983; Vernia *et al.* 1984). In the present investigation, however, both *Plantago ovata* preparations increased acetate concentration in caecal contents as well as in faeces, whereas wheat bran did not. This effect is still more pronounced when values were expressed as total faecal excretion or related to total caecal contents. On this basis, wheat bran showed a tendency towards an increased SCFA output but results are generally not significantly different from those of the fibre-free control rats, confirming other findings (Walter *et al.* 1986). Caecal acetate concentration with the husk-containing diet was higher than that with the seed-containing diet, which may result from a higher fermentation but may also be the consequence of a delayed absorption due to a higher viscosity of the intestinal contents. Thus, the acetate results presented here suggest that wheat bran has a low digestibility and *Plantago ovata* preparations have a higher digestibility, but a differentiation between husks and seeds is not possible.

pH

A high SCFA concentration in the caecum is expected to correlate with a low pH, but the converse was the case in our experiments. Rats fed with the high-wheat-bran diet had the lowest acetate concentration and by far the lowest pH in caecal contents; rats fed with the 100 g husks/kg diet had the highest caecal acetate concentration and the highest pH. As some dietary fibres induce a shift in the acetate, propionate and butyrate ratios (Topping *et al.* 1988), acetate alone may not be fully representative. There was, however, nearly no difference in the SCFA composition of cultured colonic contents from cellulose or 'psyllium'-husk-fed monkeys (Costa *et al.* 1989). In addition, the relationship between pH and total SCFA is far from being clear. Some authors have found the expected correlation (Demigné & Rémésy, 1985), some authors' findings concur with the present results (Ruppin *et al.* 1980), and some do not observe any relationship at all (Argenzio & Southworth, 1975). It is, however, a rather consistent finding that wheat bran lowers caecal pH (Jacobs & Lupton, 1982) despite a low fermentation. Less than 1% of the SCFA are protonated

at a neutral pH, but more than half of them are absorbed in the undissociated, lipid-soluble form. As a proton donor, an electroneutral $\text{Na}^+\text{-H}^+$ exchange and hydration of carbon dioxide within the lumen fluid with subsequent dissociation into proton and bicarbonate seems to be involved (Rechkemmer *et al.* 1988). The latter results in bicarbonate accumulation within the lumen and this may explain an increasing pH with increasing SCFA absorption.

β -Glucuronidase

This is an enzyme produced by a variety of bacterial strains in the large intestine (Gadelle *et al.* 1985). Its activity is strongly influenced by the diet, being high in a high-meat diet and usually lower in a high-fibre diet (Reddy *et al.* 1974; Goldin *et al.* 1978; Shiau & Chang, 1983). In the present study, the faecal and caecal β -glucuronidase activity was pronouncedly decreased by *Plantago ovata* seeds and husks, and by wheat bran to a lesser extent (faeces) or not at all (caecal contents). Total excretion was also reduced by both *Plantago ovata* preparations, while wheat bran had no effect. The importance of β -glucuronidase activity is based on its function of deconjugating many endogenous and exogenous compounds. As glucuronidation is a major detoxification mechanism in the liver and also in the intestinal mucosa, a high β -glucuronidase activity in the large intestine will release significant amounts of toxic agents excreted with bile which may affect the large intestinal mucosa. In fact, an increase in β -glucuronidase activity accompanies an increased colo-rectal tumour incidence in experimental carcinogenesis studies (Bauer *et al.* 1979; Sun & Li, 1988) which can be suppressed by a β -glucuronidase inhibitor (Takada *et al.* 1982). *Plantago ovata* husks have also been shown to possess anticarcinogenic properties in 1,2-dimethylhydrazine (DMH)-induced tumours in rats (Wilpart *et al.* 1987).

Bile acids

Conjugated bile acids are physiological substrates for β -glucuronidase in the large bowel, and bile flow is an important factor in modulating β -glucuronidase activity (Robertson *et al.* 1982; Head *et al.* 1988). It seems, however, unlikely that the *Plantago ovata* diets reduced β -glucuronidase activity by decreasing bile flow, as total bile acids in the caecum and also excretion in faeces is pronouncedly enhanced. Other possibilities are via a more direct effect on the composition or metabolic state of the microflora. Deconjugated primary bile acids are easily metabolized by the bacterial flora to the more toxic secondary ones (Morotomi *et al.* 1979; Hirano *et al.* 1981). The latter are known to increase mucosal permeability (Goerg *et al.* 1980), to stimulate mucosal proliferation (Craven *et al.* 1986) and to possess a tumour-promoting activity (Owen *et al.* 1987). By binding of bile acids and suppression of bacterial β -glucuronidase activity, the *Plantago ovata* fibres may decrease the toxicity and absorption of bile acids in the colon.

Increased bile acid excretion seems to be a main mechanism for the plasma cholesterol-reducing effect of some fibres. A hypocholesterolaemic effect has been shown for the husks (Forman *et al.* 1968; Danielsson *et al.* 1979; Burton & Manninen, 1982) and may be expected for the seeds, too, on the basis of the present results.

Morphological changes

There were no changes in the intestine with wheat bran in the present study, which confirms previous findings (Vahouny *et al.* 1985). In contrast, *Plantago ovata* husks caused a pronounced increase in small intestine and colon lengths, as well as tissue weights in the ileum, caecum and colon. Histologically, an increase in the mucosal thickness of the distal small intestine as well as an increase in villous height has been found with *Plantago ovata* husks (Sud *et al.* 1988). The seeds had almost no effect in the small intestine, but influenced

colon length to a similar degree to that of the husks; tissue weight was unaffected except for the caecum. The swelling properties of the fibres in the intestine and, thereby, the volume of the contents seem to be important factors in influencing the bowel wall (Wyatt *et al.* 1988). *Plantago ovata* husks have the highest water-binding capacity and obviously swell early in the gastrointestinal tract, whereas bulking seems to be delayed in the seeds, being evident especially in the large intestine. Wheat bran with a low water-binding capacity causes only a weak bulking of intestinal contents.

Besides the influence of an increased bulk of contents, there are additional aspects in the large intestine which may be important for the enlargement and tissue hypertrophy. These derive from the metabolic effects of the SCFA produced from fermentable fibres. It is well known that SCFA stimulate mucosal proliferation and it has been suggested that the trophic action of the SCFA is necessary to maintain the normal epithelial cell proliferation in the intestine (Sakata, 1986, 1987, 1988). In vitro studies with butyrate demonstrate an induction of cell differentiation (Muller *et al.* 1986), stimulation of the DNA repair capacity (Nishigori & Takebe, 1987), and growth delay of malignant cells (Kim *et al.* 1980). Thus, SCFA produced by *Plantago ovata* seeds or husks may contribute to the structural and functional integrity of the mucosa. Induction of mucosal cell proliferation by dietary fibres as compared with long-term fibre-free diets appears as a process of normalization and not as a pathological finding.

Mucosal enzyme activities

Finally, in the present study, *Plantago ovata* seeds and husks as well as wheat bran influenced mucosal enzyme activities in the proximal and the distal small intestine. There was a pronounced inhibition of AP, LAP, sucrase and α -amylase in the jejunum with all three dietary fibres to a similar extent. In the ileum, enzyme activities in the control group were usually lower and the effects of the fibres more heterogeneous. Enzyme studies on the proximal small intestine mucosa of rats fed other fibres show a wide spectrum of influences including stimulation and inhibition of activities (Farness & Schneeman, 1982; Johnson *et al.* 1984; Brown *et al.* 1979; Johnson & Gee, 1986). The causes of the influence of dietary fibres on mucosal enzyme activities are not well known. A direct interaction due to binding or the presence of enzyme inhibitors, especially in natural fibres, as has been shown in in vitro studies (Dunaif & Schneeman, 1981; Hansen, 1987), is unlikely. As substrate availability is important, one may suppose that dilution of the diet by the fibre may determine enzyme activities, but our experiments demonstrate that the degree of inhibition was different for the various enzymes and generally independent of dilution by fibres. In addition, their physicochemical properties, resulting in different viscosities of the contents, do not seem to play an essential role in the observed effects. It has been suggested that reduction in villous enzyme activities may be due to an increase in cell proliferation and cell-turnover induced by some fibres (Brown *et al.* 1979; Johnson & Gee, 1986), but proliferation effects, if present, generally are more evident in the distal than in the proximal part of the small intestine. In addition, stimulation of certain enzymes was observed simultaneously with the inhibition of others (Johnson & Gee, 1986; present results) which speaks against a general effect of increased cell turnover. Finally, it has been shown that fibres, e.g. *Plantago ovata* husks and seeds, also stimulate enzyme activities in vitro (Leng-Peschlow, 1990). The physiological significance for digestion of the alterations in mucosal or lumen enzyme activities by dietary fibres still remains unclear.

According to an expert panel, the fibre composition of a well-balanced diet consisting of a wide variety of foods is 700–750 g insoluble fibres and 250–300 g soluble fibres/kg (Pilch, 1987). It is not known whether this is an ideal ratio, but it might be reasonable to assume that supplementation of fibre-deficient diets with concentrated fibres should be oriented to

a healthy natural diet, if there are no special requirements. Regarding the fibre composition, *Plantago ovata* seeds may, therefore, be a suitable physiologically-acting fibre supplement. The present results indicate that the seeds are a partly-digestible dietary fibre which acts mainly in the large intestine and increases stool volume by a mixture of indigestible residue, water-binding and increase in bacterial mass. Metabolic and mucosa-protective effects based on augmented bile acid excretion, reduced β -glucuronidase activity and stimulation of SCFA production are probable. A high bulking in the upper gut, which is characteristic for *Plantago ovata* husks, is avoided.

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