

Comparative gastrointestinal and plasma cholesterol responses of rats fed on cholesterol-free diets supplemented with guar gum and sodium alginate

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The present study investigated the digestion and cholesterol-lowering effects of the water-soluble NSP guar gum (GG) and sodium alginate (SA) in laboratory animals. Groups of five male Wistar strain rats were fed semi-purified cholesterol-free diets containing 0, 50 or 100 g NSP source/kg for 21 d which comprised a 14-d adaptation period followed by a 7-d balance period. Weight gain over the balance period and food conversion ratio decreased linearly with increasing NSP intake ($P = 0.006$ and $P = 0.07$ respectively). DM digestibility decreased with increasing NSP intake ($P = 0.001$) and this effect was greater for SA-containing diets compared with GG-containing diets ($P = 0.001$). At the lower inclusion rate, 0.9–1.0 of the additional NSP was digested, but this value fell to 0.8 for both NSP sources at the 100 g/kg inclusion rate, implying that the capacity for near complete digestion of the test NSP had been exceeded. Intestinal tissue mass was increased in response to inclusion of both NSP sources. Caecal digesta pH decreased linearly with additional GG, but increased slightly with consumption of SA. Total caecal short-chain fatty acid concentrations ($\mu\text{mol/g}$ caecal contents) increased markedly with 50 g GG/kg but did not increase further with 100 g GG/kg, and were slightly lower than control values in rats consuming SA. Plasma cholesterol concentration fell linearly ($P = 0.03$) with increasing NSP in the diet and the effect was similar for both GG and SA. Total output of faecal bile acids rose in rats fed 50 g GG/kg and 50 g SA/kg ($59 \mu\text{mol/7 d}$ v. $24 \mu\text{mol/7 d}$ for control rats) with no further increase at the higher inclusion rate. These results show that SA has a strong hypocholesterolaemic effect in rats which is similar to that of GG, and that this effect is most likely to be mediated through an interruption in the entero-hepatic circulation of bile acids and not through increased hepatic supply of propionate from fermentation of the NSP in the large bowel.

Sodium alginate: Cholesterol metabolism: Bile acids: NSP digestion: Caecal fermentation

Previous studies in man and with laboratory animals have shown that consumption of moderate amounts of soluble dietary NSP may produce beneficial changes in lipid metabolism including lowered fasting blood cholesterol concentrations and alterations in the ratio of HDL:LDL-cholesterol in plasma (Tredger *et al.* 1991; Frape & Jones, 1995; Truswell, 1995; Brown *et al.* 1999). The mechanisms of these effects are not clear, but may include long-term changes in lipid metabolism in response to modified postprandial hormone levels. For example, reduced postprandial plasma insulin concentrations and attenuated gastric inhibitory peptide release as a consequence of

delayed glucose and fat absorption may influence the clearance of triacylglycerols and the transfer of cholesterol from HDL to LDL (Morgan *et al.* 1993; Frape & Jones, 1995). Other suggested mechanisms for the effects of NSP on fasting plasma cholesterol concentrations include interruption of the entero-hepatic circulation of bile acids leading to increased liver sterol output and faecal bile acid excretion (Story *et al.* 1997), and influences on the production and absorption of short-chain fatty acids (SCFA), especially propionate in the large bowel (Chen *et al.* 1984).

Alginates, extracted using acid and alkali from brown

Abbreviations: GG, guar gum; 5 %GG, 10 %GG, 50 and 100 g guar gum/kg respectively; SA, sodium alginate; 5 %SA, 10 %SA, 50 and 100 g sodium alginate respectively; SCFA, short-chain fatty acid.

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seaweed, are widely used in small amounts in foods as gelling agents, thickeners, stabilisers or emulsifiers (Michel & Macfarlane, 1996). The majority of algal polysaccharides are resistant to mammalian gastrointestinal hydrolases and their digestion is dependent on microbial depolymerisation and fermentation in the large intestine. The main constituents of alginates are uronic acids (1,4- β -mannuronic acid and 1,4- α -guluronic acids (Martin, 1986)), giving the NSP physico-chemical characteristics similar to those of pectin (galacturonic acid). Sodium alginate (SA) is readily water-soluble and produces a highly viscous gel in solution containing partially-ionized carboxyl groups. Alginates have potential for use as a 'functional food', since the production of β -elimination products by bacterial alginate-degrading enzymes may increase the growth of beneficial *Bacteroides* species of bacteria in the gut (Michel & Macfarlane, 1996). Guar gum (GG) produces the highest viscosity of any natural gum (BeMiller & Whistler, 1996), and is a galactomannan composed of a main chain of 1,4 β -D-mannopyranosyl with single unit α -D-galactopyranosyl branches.

The aim of the present experiment was to investigate the effect of SA on digestion and cholesterol metabolism in the laboratory rat in comparison with GG, a NSP with known hypocholesterolaemic properties. The experiment formed part of a series of studies which compared the physiological and nutritional effects of GG with algal dietary fibres in human volunteers (Roper *et al.* 1996). Preliminary data from the experiment have been published in abstract form (Seal & Mathers, 1996).

Materials and methods

Animals and diets

Twenty-five male Wistar strain rats, initial average weight 127 g (SE 1.2), were obtained from the Comparative Biology Centre, University of Newcastle, and housed in individual plastic metabolism cages in a controlled-environment room in this Centre throughout the study. All procedures were conducted in accordance with Home Office regulations. Rats were randomly allocated to one of five semi-purified cholesterol-free diets containing 0, 50 or 100 g GG/kg diet (Control, 5 %GG and 10 %GG respectively) or 50 or 100 g SA (5 %SA and 10 %SA respectively)/kg diet. The added NSP sources replaced some of the maize starch in the diets, which otherwise remained of constant composition and were formulated to meet all macro- and micronutrient requirements (Table 1). Cr_2O_3 (2 g/kg diet) was included as an indigestible marker. Animals were offered 15 g air-dry food at 10.00 hours daily and uneaten food was removed the following morning. Water was available *ad libitum*. The rats were fed the diets for a total of 21d, which comprised a 14-d adaptation period followed by a 7-d balance period. Food residues were removed each day, dried at 100°C and weighed.

Measurements

Animals were weighed at the beginning and end of the balance period. Complete collections of urine (into flasks

Table 1. Formulation (g/kg) of experimental diets

Diet...	Control	5 % GG	10 % GG	5 % SA	10 % SA
Ingredients					
Maize starch	250	200	150	200	150
Sucrose	288	288	288	288	288
Guar gum*	0	50	100	0	0
Sodium alginate†	0	0	0	50	100
Casein	200	200	200	200	200
Groundnut oil	150	150	150	150	150
Cellulose‡	50	50	50	50	50
Mineral premix§	40	40	40	40	40
Vitamin premix¶	20	20	20	20	20
Cr_2O_3	2	2	2	2	2
Analysed composition (g/kg as fed)					
Total N	24	26	26	27	25
Total NSP	51	91	127	93	138

5 % GG, 10 % GG, 50 and 100 g guar gum/kg respectively; 5 % SA, 10 % SA, 50 and 100 g sodium alginate/kg respectively.

* Sigma Chemical Poole, Dorset.

† Provided by CEVA, Pleubian, France.

‡ Alphacel (ICN Biomedicals Inc., Aurora, OH, USA).

§ AIN Mineral Mixture 76 (ICN Biomedicals Inc.).

¶ AIN Vitamin Mixture 76A (ICN Biomedicals Inc.).

containing 5 ml 0.05% (v/v) H_2SO_4) and faeces were made daily during the balance period. Pooled urine samples from each rat were diluted to 250 ml with double-distilled water and a portion was retained for analysis. Faecal samples were bulked, freeze-dried and milled for analyses. At the end of the balance period gastrointestinal and blood samples were obtained from all rats following terminal anaesthesia by intraperitoneal injection of Hypnorm/Midazolam (1 ml/300 g body weight, prepared by the Comparative Biology Centre). Blood samples (5 ml) were collected from the heart into heparinised syringes and cooled on ice. Plasma was separated by centrifugation and stored at -20° . The entire gastrointestinal tract was removed and the length of the small intestine measured from the pylorus to the ileo-caecal junction. The caecum and colon were separated and weighed, and the pH of the caecal contents was measured by insertion of a microelectrode connected to a pH meter. Duplicate portions (approximately 0.5 g) of caecal digesta were mixed (2:1 w/v) with deproteinising solution (H_3PO_3 (20 g/l) containing 50 mM-3-methyl-valeric acid) and stored frozen at -20° . Caecal and colonic tissue were rinsed free of digesta with physiological saline (9 g NaCl/l) lightly blotted dry and re-weighed.

Analytical and statistical methods

SCFA in caecal contents were measured by GC as previously described (Mathers *et al.* 1990). The Cr content of freeze-dried faecal samples was determined by atomic absorption spectrophotometry after ashing and wet digestion with MgSO_4 and KBrO_3 as previously described (Mathers *et al.* 1990). The N content of diluted urine and freeze-dried faecal samples was determined by combustion using a Leco FP428 nitrogen analyser (Leco Instruments (UK) Ltd, Stockport, Cheshire, UK). Plasma total cholesterol concentration was determined enzymically using a commercial kit (Ultimate 2; Roche Diagnostics, Welwyn

Garden City, Herts) on a Cobas Mira Clinical Analyser (Roche Diagnostics, Welwyn Garden City, Herts). Faecal total bile acids were determined enzymically by means of a commercial kit based on 3 α -hydroxysteroid dehydrogenase (Enzabile; Nycomed (UK) Ltd, Birmingham, UK) after methanol extraction from freeze-dried faecal samples as described by Setchell *et al.* (1983). Total bile acids determined by this method include steroids of the C₁₉, C₂₁ and C₂₄ series, including taurine and glycine conjugates of the C₂₄ series. Dietary and faecal NSP were analysed colorimetrically after enzymic digestion and acid hydrolysis as described by Englyst & Cummings (1984).

DM digestibility was calculated using the following formula:

$$\frac{\text{DM consumed} - \text{DM in faeces}}{\text{DM consumed}}$$

The apparent digestibility of added NSP was calculated using the following formulas:

NSP intake from test NSP source (A)

$$\begin{aligned} &= \text{DM intake on test diet (g/7 d)} \\ &\times (\text{NSP content of test diet} \\ &- \text{NSP content of control diet (g/kgDM)}), \end{aligned}$$

faecal NSP output from test NSP source (B)

$$\begin{aligned} &= \text{total NSP output on test diet} \\ &- (((\text{DM intake on test diet (g/7 d)} / \\ &\text{mean DM intake on control diet})) \\ &\times \text{mean NSP output on control diet}), \end{aligned}$$

digestibility of added NSP = (A - B)/A.

Statistical analysis of the difference between means for each treatment was by ANOVA using the general linear models procedure (Minitab, State College, PA, USA). Treatment sums of squares were subdivided into four *a priori* orthogonal contrasts: contrast 1, linear (L) effect of added NSP; contrast 2, deviations from linear (D) effect of added NSP; contrast 3, L \times NSP source interaction; and contrast 4, D \times NSP source interaction. Each contrast was tested against the 'between rats within diets' error term with 18 df. Tables of results show mean values with probability values for each contrast. For NSP digestibilities only, data were examined for effects of test NSP sources, dose and source \times dose interaction using a 2 \times 2 factorial analysis.

Results

Food intake, rat growth, food conversion ratio and nitrogen balance

Owing to large food refusals during the adaptation period, two rats from the 10 %SA diet were removed from the experiment. Food refusals were negligible during the balance period and DM intake was, therefore, similar across all five diets (Table 2). Weight gain during the balance period was greatest for rats consuming the control diet and decreased linearly ($P = 0.006$) with additional GG and SA. Food conversion ratio (g food DM eaten/g live-weight gain) was also lower for GG- and SA-containing diets and decreased linearly with increasing GG and SA in the diet ($P = 0.07$; Table 2). DM digestibility decreased linearly with increasing NSP intake ($P < 0.001$; Table 2) with a steeper slope for SA-containing diets compared with GG-containing diets ($P = 0.001$; contrast 3). The slightly higher concentration of N in GG- and SA-containing diets (Table 1) was reflected in higher N intakes for animals consuming these diets compared with the control group. Faecal N output increased linearly ($P < 0.001$) with increasing NSP intake to reach approximately double control levels with the

Table 2. Food DM intake, indigestibility, growth rate and aspects of nitrogen metabolism during balance period in rats given semi-purified diets' supplemented with 50 and 100 g guar gum/kg (5 % GG and 10 % GG respectively) or 50 and 100 g sodium alginate/kg (5 % SA and 10 % SA respectively)*

(Means for five rats per diet, except 10 % SA where values are for three rats per diet)

Diet...	Control	5 % GG	10 % GG	5 % SA	10 % SA	SEM	Statistical significance of contrast† (P):			
							1	2	3	4
DM intake (g/7 d)	100	100	99	98	100	0.3	0.58	0.08	0.89	0.007
Final rat wt (g)	234	227	214	216	210	3.0	0.006	0.79	0.68	0.18
Wt gain (g/7 d)	36	31	29	31	27	1.5	0.07	0.69	0.70	>0.99
Food conversion ratio (g DM consumed/wt gain (g))	0.36	0.31	0.29	0.31	0.27	0.002	0.07	0.75	0.69	0.89
Faecal DM output (g/7 d)	7.9	10.2	11.4	9.3	13.8	0.42	<0.001	0.38	<0.001	0.07
DM digestibility	0.92	0.89	0.89	0.91	0.86	0.002	<0.001	0.51	0.001	0.15
N intake (g/7 d)	2.53	2.75	2.73	2.72	2.64	0.019	<0.001	<0.001	0.001	0.17
Faecal N output (g/7 d)	0.14	0.26	0.32	0.19	0.25	0.015	<0.001	0.81	0.35	0.12
Urinary N output (g/7 d)	0.93	0.93	0.99	0.82	0.91	0.024	0.50	0.18	0.35	0.12
N retention (g/7 d)	1.46	1.56	1.42	1.72	1.48	0.030	0.37	<0.001	0.49	0.03

* For details of diets and procedures, see Table 1. and p. 318.

† Contrast 1, linear (L) effect of added NSP; contrast 2, deviations from linear (D) effect of added NSP; contrast 3, L \times NSP source interaction; contrast 4, D \times NSP source interaction.

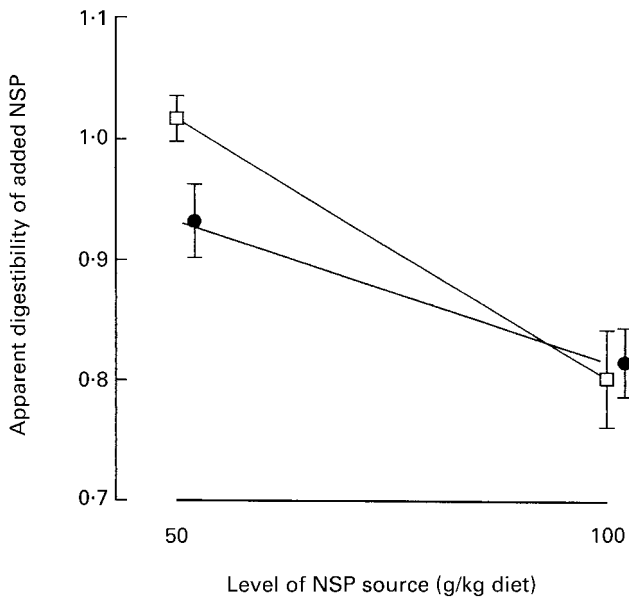


Fig. 1. Mean apparent digestibility of added test NSP sources in rats fed semi-purified diets supplemented with 50 and 100 g guar gum/kg (□) or 50 and 100 g sodium alginate/kg (●). For details of diets and procedures, see Table 1 and p. 318. Values are means with their standard errors represented by vertical bars for five rats per diet except 100 g sodium alginate/kg where there were three rats per diet. The effect of the level of added NSP was significant ($P < 0.001$). For details of calculations see p. 319.

higher dose of NSP. Urinary N excretion was not significantly influenced by diet but there was a significant deviation from linearity in the effect of added GG and SA ($P < 0.001$) on N retention, which was highest at the 50 g/kg inclusion rate for both NSP sources.

Apparent digestibility of the added test NSP sources is shown in Fig. 1. With 50 g GG/kg diet, the added NSP was completely digested (95 % CI 1.08, 0.95) and approximately 0.93 (95 % CI 1.00, 0.87) of the SA was digested in the 5 % SA diet. At the 100 g/kg inclusion rate, digestibility of the added NSP fell significantly ($P < 0.001$) to 0.81 (95 % CI 0.86, 0.76) for both test NSP sources.

Intestinal tissue mass, caecal pH and fermentation pattern

Small intestine length of GG- and SA-fed rats increased by approximately 20 % compared with control rats (Table 3). There was a significant linear increase in caecal tissue mass and the mass of caecal digesta in response to inclusion of the NSP sources which was similar for both GG and SA ($P < 0.001$; Table 3) in response to inclusion of the NSP. Colonic digesta mass and colonic tissue mass were higher in animals fed GG and SA with similar values for 50 and 100 g/kg inclusion rates for both NSP sources.

Caecal digesta pH fell steadily from 6.6 on the control diet to 6.1 in rats consuming the 10 % GG diet, but was slightly increased above the value for the control diet by consumption of diets containing SA (Table 4). Total SCFA ($\mu\text{mol/g}$ caecal contents) increased markedly with the 5 % GG diet but did not increase further with the 10 % GG diet. Total SCFA concentrations in caeca of rats consuming SA were slightly lower than that of control rats and was unaffected by the level of inclusion of SA. Adding 50 g GG/kg diet produced a 60 % increase in caecal butyrate molar proportion, which was balanced by reductions in the molar proportions of all other measured SCFA (Table 4). However, with the higher inclusion rate for GG, the proportion of butyrate and other SCFA returned to similar to control values. Consumption of SA-containing diets was associated with a modest increase in the proportion of acetate with compensatory decreases in the molar proportions of propionate and, to a lesser extent, of butyrate. The contributions made by both isobutyrate and valerate to the SCFA pool were reduced linearly ($P = 0.003$ and $P < 0.001$ respectively) by inclusion of increasing doses of GG and SA in the diet. In contrast, there were no significant between-diet effects on the molar proportions of isovalerate.

Plasma cholesterol concentrations and output of faecal bile acids

Plasma total cholesterol concentration fell linearly ($P = 0.03$; Fig. 2(a)) with increasing NSP in the diet and the effect was similar for both GG and SA. Output of total bile acids during the balance period rose from 24 $\mu\text{mol/7 d}$

Table 3. Rat weight, small intestine length, caecal and colonic organ mass, caecal and colonic tissue weights for rats given semi-purified diets supplemented with 50 and 100 g guar gum/kg (5 % GG and 10 % GA respectively) or 50 and 100 g sodium alginate/kg (5 % SA and 10 % SA respectively)*

(Means for five rats per diet, except 10 % SA where values are for three rats per diet)

Diet...	Control	5 % GG	10 % GG	5 % SA	10 % SA	SEM	Statistical significance of contrast† (P):			
							1	2	3	4
Small intestine length (cm)	107	129	124	121	123	2.1	0.002	0.003	0.96	0.08
Caecum										
Total mass (g)	2.30	4.11	5.56	4.67	5.75	0.314	<0.001	0.29	0.78	0.35
Tissue mass (g)	0.53	0.85	1.02	0.78	1.01	0.044	<0.001	0.46	0.85	0.41
Digesta mass (g)	1.77	3.26	4.53	3.88	4.74	0.274	<0.001	0.29	0.74	0.25
Colon										
Total mass (g)	2.02	3.07	2.93	3.37	3.45	0.143	0.002	0.006	0.16	0.35
Tissue mass (g)	0.96	1.13	1.24	1.14	1.28	0.046	0.03	0.75	0.78	0.96
Digesta mass (g)	1.06	1.94	1.69	2.35	2.18	0.116	0.004	0.001	0.09	0.23

* For details of diets and procedures, see Table 1 and p. 318.

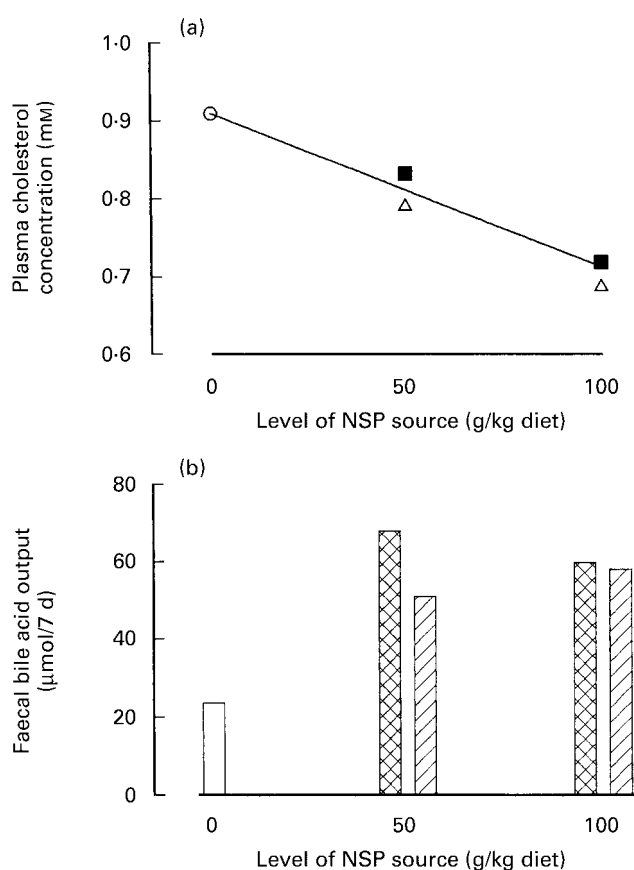
† Contrast 1, linear (L) effect of added NSP; contrast 2, deviations from linear (D) effect of added NSP; contrast 3, L × NSP source interaction; contrast 4, D × NSP source interaction.

Table 4. pH, total short-chain fatty acid (SCFA) concentration ($\mu\text{mol/g}$ caecal contents) and molar proportions ($\mu\text{mol}/100 \mu\text{mol}$) of individual SCFA in the caeca of rats given semi-purified diets supplemented with 50 and 100 g guar gum/kg (5 % GG and 10 % GG respectively) or 50 and 100 g sodium alginate (5 % SA and 10 % SA respectively)*

(Means for five rats per diet, except 10 % SA where values are for three rats per group)

Diet...	Control	5 % GG	10 % GG	5 % SA	10 % SA	SEM	Statistical significance of contrast† (P):			
							1	2	3	4
Caecal pH	6.6	6.3	6.1	6.8	6.7	0.06	0.002	0.003	0.96	0.08
Total SCFA ($\mu\text{mol/g}$ caecal content)	79	102	106	62	70	4.28	0.07	0.46	0.001	<0.001
Molar proportions of individual SCFA										
Acetate	64.1	61.4	68.3	71.0	71.7	1.39	0.10	0.81	0.43	0.02
Propionate	19.9	17.5	18.5	16.2	15.1	0.79	0.30	0.29	0.23	0.58
Isobutyrate	1.7	1.2	0.6	0.8	1.0	0.12	0.003	0.32	0.19	0.28
Butyrate	10.9	17.4	10.2	9.5	9.7	1.10	0.55	0.15	0.89	0.02
Isovalerate	1.8	1.5	1.8	1.4	1.7	0.10	0.98	0.15	0.84	0.88
Valerate	1.6	1.0	0.7	1.0	0.8	0.09	<0.001	0.30	0.60	0.76

* For details of diets and procedures, see Table 1 and p. 318.

† Contrast 1, linear (L) effect of added NSP; contrast 2, deviations from linear (D) effect of added NSP; contrast 3, L \times NSP source interaction; contrast 4, D \times NSP source interaction.**Fig. 2.** (a) Concentrations of total cholesterol in plasma from rats fed semi-purified diets supplemented with 50 and 100 g guar gum/kg (■) or 50 and 100 g sodium alginate/kg (△), (○), Control diet. For details of diets and procedures, see Table 1 and p. 318. Values are means for five rats per diet except 100 g sodium alginate/kg where there were three rats per diet. There was a significant ($P = 0.03$) linear effect of level of added NSP. The pooled SEM for an individual diet group was 0.038 mm. (b) Faecal bile acid output of the rats during the balance period. (⊗), Guar gum; (⊘), sodium alginate; (□), Control diet. There were significant linear ($P = 0.003$) and deviations-from-linear ($P = 0.02$) effects of the level of added NSP. The pooled SEM for an individual diet group was 4.63 $\mu\text{mol}/7 \text{ d}$.

in control rats to a mean of 59 $\mu\text{mol}/7 \text{ d}$ in rats fed 5 %GG and 5 %SA diets respectively, with no further increase with the higher inclusion rate (Fig. 2(b)).

Discussion

Fate of NSP sources in the gut

In rats fed 5 %GG and 5 %SA diets, 0.9–1.0 of the additional NSP was digested, confirming the high overall digestibility of these soluble NSP. Similar calculations for the 100 g/kg inclusion rate, however, show that digestibility of the additional NSP source was significantly reduced ($P < 0.001$) and approximately 0.2 of the additional NSP had not been digested, implying that at the higher inclusion rate the capacity for near complete digestion of the NSP had been exceeded. Approximately 100 % disappearance of guar gum within the alimentary tracts of rats and human subjects has been reported (Nyman *et al.* 1986), but to our knowledge the present study is the first study that has attempted to determine the effect of an oral dose of guar gum (or sodium alginate) on digestibility of the NSP in the hydrocolloid. In contrast to earlier studies in which peas (*Pisum sativum*); bread and beans (*Phaseolus vulgaris*) were fed to rats at a range of doses and where there was little evidence of a diminution in digestibility of NSP with higher intakes (Goodlad & Mathers, 1990, 1992; Key & Mathers, 1993a,b, 1995), the present study provided clear evidence that with 100 g NSP source/kg diet, there was significantly ($P < 0.001$) reduced whole-gut NSP digestibility (Fig. 1).

Most algal polysaccharides are resistant to digestion by digestive enzymes, and almost all uronic acids derived from sodium alginate consumed by ileostomy patients could be recovered in ileostomy fluid (Sandberg *et al.* 1994). *In vitro* fermentation of alginates using human faecal flora (Michel *et al.* 1996) showed that approximately 83 % of the fibre disappeared, but only 57 % was metabolised to SCFA, resulting in a small change in pH of the incubation medium. Michel *et al.* (1996) concluded that the disappearance of uronic acids was due to the

involvement of 'an unusual and unknown' fermentative pathway resulting in approximately 40 % of the disappeared material being metabolised through pathways other than SCFA and gas production. It is possible that all, or virtually all, the NSP that disappeared within the intestines of the rats fed GG and SA in the present study did so by bacterial degradation within the large bowel. The reason for the reduced NSP digestibility with the higher NSP intake was not established, but may be associated with inadequate time for bacterial hydrolysis of the test polymers, since alterations in gut transit time has a strong influence on extent of NSP digestion (Mathers, 1991).

There was a strong linear increase in faecal N output with increasing consumption of GG and SA, which is consistent with previous observations in rats fed increasing levels of dietary NSP (for example, see Goodlad & Mathers, 1990; Key & Mathers, 1993a). It has been suggested that increased faecal N output may be due to enhanced loss of endogenous N arising from stimulation of mucosal cell turnover (Skurpakkar *et al.* 1979). Although tissue hypertrophy was observed in the present experiment with both NSP sources, no measurements of intestinal mucosal cell proliferation were made. Fairweather Tait *et al.* (1983) have demonstrated that, although faecal N loss was increased in bean-fed rats, this loss could not be attributed to increased cell turnover. An alternative source of faecal N is bacterial N, which may be elevated in response to increased supply of fermentable organic matter to the caecal or colonic bacteria (Mason & Palmer, 1973; Goodlad & Mathers, 1990, 1992; Key & Mathers, 1993b). Fermentation of supplemental GG and SA would be expected to increase bacterial cell growth in the large bowel (Key & Mathers, 1995) and to elevate bacterial N output in faeces.

Hypocholesterolaemic effects of NSP sources

Many studies with human volunteers have investigated the hypocholesterolaemic effects of NSP from a variety of sources including oats, psyllium, pectin and guar gum. A recent meta-analysis of data from sixty-seven controlled trials (Brown *et al.* 1999) concluded that the cholesterol-lowering effects of oats, psyllium and pectin were similar. The authors were unable to compare the effects of GG, owing to the limited number of studies available in which GG intakes were similar to those of the other NSP. Whilst the effects of the NSP were significant, they were considered small (-0.045 mmol/l per g 'soluble fibre') within the 'practical' range of NSP intake (2–10 g/d). The data from animal studies, using a variety of laboratory rodents and larger animals, have also shown consistently that soluble NSP have a strong cholesterol-lowering effect, although the majority of these studies have included NSP at levels that could not be achieved readily in Western diets, and differences in lipid metabolism between species mean that direct comparison with human subjects should be made with some caution. In the present study there was a similar linear decrease in plasma total cholesterol concentration with both NSP sources with, on average, a 14 % reduction in cholesterol concentration for the higher dose of the two NSP sources compared with control rats. This reduction in plasma cholesterol concentration was achieved despite

feeding cholesterol-free diets, and must therefore reflect changes in endogenous cholesterol metabolism.

The mechanisms by which NSP, which form viscous aqueous solutions, exert their hypocholesterolaemic effects remain unclear. The NSP may exert direct physico-chemical effects within the small intestine which interfere with formation and diffusion of bile acid and cholesterol-containing micelles through the viscous matrix of the digesta. This immobilization may result in reduced absorption of cholesterol and bile acids (Gallaher *et al.* 1993; Carr *et al.* 1996) causing an interruption in the entero-hepatic circulation of cholesterol and its metabolites. The resulting decrease in the cholesterol content of liver cells leads to an up regulation of LDL receptors and thus increased clearance of LDL-cholesterol from the blood (Fernandez, 1995). Changes in the activity of cholesterol 7 α -hydroxylase has also been reported in rats fed soluble NSP (Buhman *et al.* 1998) or cholestyramine, a bile acid sequestrant (Chiang *et al.* 1990). The increased faecal excretion of total bile acids observed in rats fed GG and SA (Fig. 2(b)) is consistent with studies in laboratory rats fed cholesterol-free diets supplemented with a range of soluble NSP including GG at similar inclusion rates to those used in the present study (Gallaher *et al.* 1992; Overton *et al.* 1994; Moundras *et al.* 1997; Buhman *et al.* 1998). Studies in animals fed cholesterol-containing diets (for example, see Trautwein *et al.* 1998, 1999), and in human subjects fed normal diets (Jenkins *et al.* 1980; Bosaeus *et al.* 1986), have also reported increased faecal bile acid excretion following consumption of soluble NSP. Approximately a twofold increase in neutral sterol output (not determined in the present experiment) has been observed in rats fed cholesterol-free diets containing 75 g GG/kg (Moundras *et al.* 1997) and 50 g psyllium husk/kg (Buhman *et al.* 1998), and a similar increase in endogenous sterol loss may also contribute to the cholesterol-lowering effect of both GG and SA observed in the present experiment. There are variable responses to different NSP sources (Story *et al.* 1997), with some NSP (e.g. oat bran and psyllium) causing a much greater increase in faecal bile acid output than other sources (e.g. barley bran). Conflicting results on the effects of alginates between laboratory animals and human subjects have been reported. For example, Sandberg *et al.* (1994) found that bile acid excretion was reduced in ileostomy patients consuming sodium alginate, whereas Wu & Peng (1997) reported that faecal bile acid output was increased in rats fed diets containing 50 g alginate/kg. The differences between responses in human subjects and laboratory animals may be due to the higher levels of NSP intake used in the latter which are not achievable in human subjects, and to differences in lipid metabolism between species. In the present experiment the effects of GG and SA were similar, with a two- to threefold increase in faecal bile acid output compared with control rats. This increase was observed at the 50 g/kg level of added NSP source, but did not increase further when the NSP source was added at 100 g/kg of the test diets, despite the fact that the linear fall in plasma cholesterol concentration continued to the NSP source inclusion level of 100 g/kg. This finding suggests that, while the interruption in the entero-hepatic circulation of bile acids may be important in reducing

plasma cholesterol concentration at lower intakes of the test NSP sources, another explanation must be sought for the additional fall in plasma cholesterol concentration between 50 and 100 g NSP source/kg diet.

Is increased caecal propionate responsible for cholesterol lowering?

Since altered sterol absorption does not provide an adequate explanation for the hypercholesterolaemic effects of viscous NSP sources, alternative mechanisms have been sought. Anderson (1985) suggested that increased hepatic propionate supply via the portal vein from large-bowel NSP fermentation may inhibit hydroxymethylglutaryl-CoA reductase, the rate-limiting enzyme for cholesterol biosynthesis (Rodwell *et al.* 1976; Levrat *et al.* 1994), and thus reduce circulating cholesterol concentrations. There is no doubt that increased consumption of fermentable NSP has been associated with increased caecal production of SCFA, and higher concentrations of SCFA, including propionate, in portal blood (Goodlad & Mathers, 1990). However, the reported effects of increased propionate availability on blood cholesterol concentrations or on hepatic cholesterol synthesis rate are not consistent (Chen *et al.* 1984; Illman *et al.* 1988; Venter *et al.* 1990; Todesco *et al.* 1991; Beaulieu & McBurney, 1992; Demigné *et al.* 1995; Lin *et al.* 1995; Hara *et al.* 1998). This apparent inconsistency, coupled with apparent variations in response between human and rat hepatocytes *in vitro* (Lin *et al.* 1995), casts some doubt on the primary role of propionate in reducing plasma cholesterol concentrations. In rats fed GG caecal propionate concentrations were higher than those for both control ($P = 0.13$) and SA-fed ($P = 0.01$) rats (18.7 mM *v.* 15.7 and 10.2 mM respectively) and this increase propionate would be expected to result in increased portal appearance of the SCFA (Goodlad & Mathers, 1990). However, in animals fed SA caecal propionate concentrations were approximately 0.66 of those in control rats and 0.55 of those in GG-fed rats. Although data on hepatic supply of propionate are not available, it seems unlikely that the hypocholesterolaemia observed in SA-fed rats was due to the effects of propionate on hepatic cholesterol synthesis.

Conclusion

The results presented here show that SA has a strong hypocholesterolaemic effect in rats (equivalent to that of GG) and that this response is most probably mediated through an interruption in the entero-hepatic circulation of cholesterol and bile acids, and not through fermentation of the NSP to produce additional propionate in the large intestine. This finding suggests that the NSP may have potential for use as a dietary supplement in human subjects at risk of developing hypercholesterolaemia.

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