Caecal and faecal short-chain fatty acids and stool output in rats fed on diets containing non-starch polysaccharides

BY C. A. EDWARDS* AND M. A. EASTWOOD

Gastrointestinal Laboratory, Edinburgh University, Western General Hospital, Edinburgh EH4 2XU

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The exact mechanisms by which non-starch polysaccharides increase stool output are unknown. In the present study the hypothesis that the site of fermentation and short-chain fatty acid (SCFA) accumulation is related to the action of non-starch polysaccharides (NSP) on stool output was tested. The basal diet (45 g NSP/kg) of forty-three male Wistar rats was supplemented with 50 g/kg of either guar, karaya, tragacanth, gellan, xanthan or ispaghula for 28 d. A further twenty-three rats were maintained on the basal diet for the same time period. Faeces were then collected over 2 d and caecal contents obtained post-mortem. Caecal and faecal wet and dry weights and SCFA were measured. Each supplement had a different effect on the caecal and faecal contents but they appeared to fall into three groups when compared with the basal diet. In group 1, guar gum affected only caecal SCFA. It had no effect on stool output or faecal SCFA. In group 2, karaya increased caecal SCFA and tragacanth, karaya and xanthan increased faecal SCFA and faecal water. In group 3, ispaghula and gellan had no consistent effect on caecal or faecal SCFA concentrations but increased total faecal SCFA output and increased faecal wet and dry weight. Although the knowledge that SCFA are rapidly absorbed in the large intestine has led us to believe that they play no role in determining faecal output, these results suggest that in some cases where NSP are slowly fermented, and increase faecal SCFA, the role of the SCFA may need to be reassessed.

Non-starch polysaccharides: Short-chain fatty acids: Caecal fermentation: Stool output

The mechanisms by which non-starch polysaccharides (NSP) increase faecal output, and in particular faecal water, are not fully understood. NSP which are resistant to fermentation by bacteria in the large intestine are the most effective faecal bulkers, probably because they retain their water-holding capacity (WHC). The extent to which a NSP is fermented in the large intestine of an animal is dependent not only on the ease of fermentation but also on the time it remains within the large intestine (Van Soest *et al.* 1982). The factors which determine colonic transit time are not clear but propulsion may be increased by distension (Chauve *et al.* 1976), bile acids (Kirwan *et al.* 1975) or by the stimulation of the mucosa with the edges of particulate matter (Tomlin & Read, 1988). The effect of fermentation products on motility has not been investigated thoroughly and short-chain fatty acids (SCFA) have been shown to inhibit motility in the caecum of the sheep (Svendsen, 1972) and the isolated rat colon (Squires *et al.* 1992), but to stimulate contractions in rat mid- and distal colonic strips (Yajima, 1985). The osmotic activity of the SCFA has been largely discounted as it has been shown that they are rapidly absorbed (McNeil *et al.* 1978). The major sites of fermentation are thought to be the caecum and proximal colon but it may be possible, if propulsion is stimulated before fermentation is complete, for fermentation of a NSP to continue further along the large intestine. If this occurs, the SCFA produced at distal sites may have a greater effect on stool output as the water remaining in the gut

* Present address: Department of Human Nutrition, University of Glasgow, Yorkhills Hospitals, Glasgow G3 8SJ.

Table 1. In vitro *fermentability and residual water-holding capacity (WHC) of non-starch polysaccharides (NSP) used in the present study**

NSP	Residual WHC† (g/g)	Fermentability† (mmol SCFA/l produced *in vitro* after 24 h)
Guar	1·87	71·4
Tragacanth	2·13	67·4
Karaya	4·65	24·2
Xanthan	2·15	63·4
Ispaghula	ND	40·0
Gellan	3·08	37·2
Control	0·91	15·5

ND, not determined; SCFA, short-chain fatty acids.
* Values from Adiotomre *et al.* (1990) and Edwards & Eastwood (1992).
† After 24 h incubation with human faeces.

is related to the balance between SCFA production and absorption. In addition, as SCFA have been shown to influence the metabolism and turnover of colonic enterocytes, higher concentrations of SCFA at more distal sites, where disease is more common, may have an important role in pathology and treatment.

The aim of the present study was to test the hypothesis that the site of SCFA accumulation is related to the action of NSP on stool output. Further, that the fermentability of NSP is related to the site of SCFA accumulation and so influences faecal output. We therefore compared the caecal and faecal SCFA of rats fed on a variety of NSP with a range of *in vitro* fermentabilities (Adiotomre *et al.* 1990; Edwards & Eastwood, 1992; Table 1) and have related these to the effects of the NSP on faecal output and faecal water.

MATERIALS AND METHODS

Animals

A total of sixty-six male Wistar rats, initial weight approximately 150 g, were fed on a basal diet containing (g/kg): NSP (measured by the Englyst method, Englyst & Cummings, 1984) 45, digestible fat 29·9, digestible protein 129, starch 629·5, sugar 22·8 (Special Diet Services Ltd., Witham, Essex) for 4 weeks before administration of the test diet. They were housed together in groups of up to five and maintained within the facilities of the Animal Unit, Western General Hospital, Edinburgh. The room was regulated to a 12 h light – 12 h dark schedule.

Diets

Rats were maintained on the basal diet for 4 weeks. Six groups of seven or eight rats were studied for the test NSP. As many rats were being studied and the number of metabolism cages was limited, the groups were staggered, with two groups being studied at any one time, and a control group at the beginning and end of the study giving a total of twenty-three control animals. After 4 weeks each group was maintained on the basal diet supplemented with 50 g/kg of either guar gum (molecular weight (M_r) $0{\cdot}25 \times 10^6$, Sigma Chemical Co. Ltd., Dorset), xanthan (Keltrol T; M_r 2×10^6, Kelco Inc., San Diego, CA, USA), karaya (M_r $4{\cdot}7 \times 10^6$, Norgine Ltd., London), tragacanth (M_r $0{\cdot}5$–1×10^6, D. M. W. Anderson, Department of Chemistry, University of Edinburgh), gellan (M_r $0{\cdot}5$–1×10^6, Kelco Inc.) or ispaghula (Richardson and Vicks Ltd, Egham, Surrey) for a further 4 weeks.

Animals were allowed to feed and drink *ad lib.* Weight gain was monitored weekly. For 3 d at the end of the test diet period the animals were housed separately in metabolism cages.

Sample collection and analysis

While the animals were housed in the metabolism cages, food intake was measured. Faeces were collected daily. In a previous study (Edwards *et al.* 1992) we have shown that for faecal pellets over 1·5 g there is only 8% water loss over 24 h in our faecal collection system independent of diet. Values for wet weight are calculated allowing for this water loss.

After 3 d the animals were killed by cervical dislocation. The caecum and complete large intestine were removed. The two were separated and the contents carefully removed. The weight of the caecal contents was measured and the contents treated in an identical manner to the faecal material. The caecal and large intestinal tissue were carefully dissected free from fat and mesentery and washed with saline (9 g NaCl/l). They were then blotted and weighed. After weighing, the pH of faeces and caecal contents were adjusted to > pH 9 before freeze-drying.

SCFA were analysed by gas chromatography of diethyl-ether extracts (Spiller *et al.* 1980). Faecal samples from the last 2 d in the metabolism cages were pooled and used for all analyses. Collections from the first day were not analysed to allow for the adaptation of the animals to the cages. Animals were placed in the metabolism cages in the morning. The faeces were collected before removing the animals on the final day. Any faeces passed during handling on the final day after removal from the cages were regarded as colonic contents. Previous studies have indicated that 2 d collections of faeces give values for stool output that are not significantly different from values obtained over longer collection periods (28 d; Edwards *et al.* 1992).

Statistical analysis

Results from rats given NSP were compared with rats given the basal diet by one-way analysis of variance followed by Student's *t* test using the pooled estimate of standard deviation.

RESULTS

Food intake, body and tissue weights

Rats fed on guar gum and gellan ingested significantly more food, calculated without the contribution of the added NSP, than animals fed on the basal diet (Table 2). There was no significant difference for the other diets (Table 2). There was no difference in the final weight of any group of rats when compared with the rats fed on the basal diet. Ispaghula-fed rats, however, had a higher final weight than some of the other groups of rats. This was due to a higher gain in body weight in the run-in period on the basal diet. The reason for this is unclear. Each analysis of the results is a comparison of each test group with the rats maintained on the basal diet and so this does not affect the overall conclusions (Table 2). Ingestion of all the NSP was associated with an increase in caecal tissue weight although this was most marked with guar and karaya. Only guar, tragacanth and ispaghula significantly increased colonic tissue weight. Guar and ispaghula increased colonic length (Table 2).

Caecal contents

The wet and dry weights of the contents of the caecum were increased by guar, tragacanth, karaya, and ispaghula: xanthan and gellan both decreased caecal content dry weight. Xanthan also decreased caecal content wet weight (Table 3).

Guar gum increased the concentration of caecal SCFA as a component of both dry and

Table 2. *Mean food intake and caecal and colonic tissue variables of rats fed on a basal diet (45 g non-starch polysaccharides (NSP)/kg) supplemented with 50 g NSP/kg from various sources, for 28 d*†

Diet	Food intake‡ (g dry wt/d)	Caecal tissue wt (g)	Colon tissue wt (g)	Colon length (mm)	Final rat wt (g)
Basal (*n* 23)	22·8§	0·77	1·50	190	390·0
Guar (*n* 7)	24·8*	1·12***	1·86*	223***	353·8
Tragacanth (*n* 7)	24·2	1·02***	1·73*	206	349·1
Karaya (*n* 7)	22·2	1·08***	1·47	190	382·6
Xanthan (*n* 7)	23·6	1·0***	1·46	204	387·1
Gellan (*n* 7)	26·9***	0·97***	1·48	191	349·7
Ispaghula (*n* 8)	22·8	0·97***	2·27***	219**	411·8
Pooled SD	1·9	0·11	0·33	20	39·6

Mean values were significantly different from those of the basal diet: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$ (Student's *t* test after ANOVA).

† For details of diets and procedures, see Table 1 and pp. 774–775.

‡ Excluding supplemented NSP.

§ *n* 18.

Table 3. *Short-chain fatty acid (SCFA) content in the caecum of rats fed on a basal diet (45 g non-starch polysaccharides (NSP)/kg) supplemented with 50 g NSP/kg from various sources, for 28 d*†

Diet	Total SCFA			Wet wt of caecal contents	Dry wt of caecal contents	Caecal pH
	μmol/g dry wt	μmol/g wet wt	μmol/g in caecum			
Basal (*n* 23)	461·7	87·6	350·3	4·00	0·75	6·56‡
Guar (*n* 7)	699·3*	113·7**	871·5***	7·58***	1·25***	6·58
Tragacanth (*n* 7)	451·8	78·0	439·7	5·63***	0·97**	6·57
Karaya (*n* 7)	467·1	89·7	611·2***	6·91***	1·31***	6·71
Xanthan (*n* 7)	448·2	67·5*	179·0**	2·73**	0·40***	6·6
Gellan (*n* 7)	585·3*	62·5**	267·8	4·24	0·45***	7·08***
Ispaghula (*n* 7)	431·8	67·3*	464·9*	6·91***	1·1***	6·38
Pooled SD	96·8	18·3	136·4	0·90	0·18	0·27

Mean values were significantly different from those for the basal diet: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$ (Student's *t* test after ANOVA).

† For details of diets and procedures, see Table 1 and pp. 774–775.

‡ *n* 18.

wet weights (Table 3). Guar also increased the total amount of SCFA in the caecum. Gellan increased SCFA measured as a component of dry weight but reduced SCFA concentration per g wet weight, and had no effect on total amount of SCFA in the caecum. Xanthan decreased the SCFA concentration per g wet weight and the total amount of SCFA in the caecum. Ispaghula also decreased SCFA concentration per g wet weight probably reflecting the increased proportion of water in the caecum. Tragacanth had no significant effect on amount or concentration of SCFA.

Tragacanth significantly reduced, whereas gellan increased, the molar proportions of acetic acid in the caecum (see Table 5). Ispaghula increased the proportion of propionic acid and decreased the proportion of butyric acid whereas gellan had the opposite effect.

Table 4. *Mean faecal output and faecal short-chain fatty acid (SCFA) content of rats fed on a basal diet (45 g non-starch polysaccharides (NSP)/kg) supplemented with 50 g NSP/kg from various sources, for 28 d*†

	Faecal output (g/d)		Faecal water (g/kg)	Faecal water (g)	Total faecal SCFA			Faecal pH
	Wet wt	Dry wt			μmol/g dry wt	μmol/g wet wt‡	μmol/d	
Basal (*n* 23)	3·26	1·58	534§	1·70	86·3	40·3	124·1	7·27‖
Guar (*n* 7)	3·85	1·61	569	2·23	91·6	39·5	153·9	6·97
Tragacanth (*n* 7)	4·65**	2·01*	564	2·64**	125·5	53·9*	248·2**	6·89*
Karaya (*n* 7)	4·92***	1·95	603**	2·97***	139·5*	55·1	273·0**	ND
Xanthan (*n* 7)	4·73**	1·86	602	2·87***	183·5***	71·3*	351·8***	6·43***
Gellan (*n* 7)	6·11***	2·68***	563	3·44***	72·7	31·7	191·3	7·04
Ispaghula (*n* 8)	5·87***	2·56***	652	3·68***	108·7	39·9	238·1**	6·69***
Pooled SD	1·10	0·48	38	0·74	39·6	13·6	87·3	0·36

ND, not determined.

Mean values were significantly different from those for the basal diet: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$ (Student's *t* test after ANOVA).

† For details of diets and procedures, see Table 1 and pp. 774–775.

‡ Assuming 8% water loss.

§ *n* 22.

‖ *n* 18.

Table 5. *Molar proportions of short-chain fatty acids (SCFA) in caecal contents and faeces of rats fed on a basal diet (45 g non-starch polysaccharides (NSP)/kg) supplemented with 50 g NSP/kg from various sources for 28 d*†

	Caecal SCFA			Faecal SCFA		
	Acetic	Propionic	N-butyric	Acetic	Propionic	N-butyric
Basal (*n* 23)	66·2	17·3	13·0	80·0	9·7	5·0
Guar (*n* 7)	64·8	16·3	15·0	85·1*	9·3	0·95***
Tragacanth (*n* 7)	61·5*	19·7	14·0	85·0*	9·6	1·7**
Karaya (*n* 7)	67·9	15·7	12·2	76·2	13·9*	3·3
Xanthan (*n* 7)	66·1	15·6	13·6	74·1*	13·3*	6·1
Gellan (*n* 7)	71·3*	11·5***	19·0***	71·3***	11·5	6·0
Ispaghula (*n* 8)	66·1	22·5**	9·4*	78·6	14·1*	5·0
Pooled SD	4·9	3·5	3·6	5·3	4·0	2·3

Mean values were significantly different from those for the basal diet: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$ (Student's *t* test after ANOVA).

† For details of diets and procedures, see Table 1 and pp. 774–775.

There was no significant effect of any of the other NSP on the molar proportions of the SCFA.

Gellan increased caecal pH (Table 3); none of the other NSP had any effect.

Faecal content

Guar gum had no effect on faeces apart from causing a slight increase in water content. Tragacanth, karaya and xanthan increased faecal wet weight but not dry weight. They increased faecal SCFA concentration (although this did not achieve statistical significance for tragacanth), increased daily SCFA output and decreased faecal pH (Table 4). Gellan

and ispaghula increased faecal wet and dry weight and ispaghula increased total SCFA output, but neither NSP had a significant effect on faecal SCFA concentrations.

Guar and tragacanth significantly increased the molar proportion of acetic acid in faeces (Table 5) whereas xanthan and gellan had the opposite effect. Karaya, xanthan and ispaghula all significantly increased the proportion of faecal propionic acid. Guar and tragacanth significantly decreased the proportion of butyric acid. None of the NSP tested significantly increased the molar proportion of butyric acid.

DISCUSSION

The NSP in the present study showed a range of effects on caecal and faecal SCFA and stool output that probably relate to their fermentability. The effects span a continuum which can be approximately divided into three groups. The first group, containing guar gum, was rapidly fermented, had the greatest effects on caecal SCFA and had little effect on faeces. Tragacanth, karaya and xanthan form the second group. These NSP had moderate effects on caecal SCFA, increased faecal SCFA concentration and increased faecal water. The final group, consisting of gellan and ispaghula, had little effect on the caecal SCFA. They had less effect on the total faecal SCFA than the second of group NSP and did not increase faecal SCFA concentration. However, they had the greatest effect on stool output increasing both wet and dry weights, probably in part due to some remaining physical structure.

The increase in stool water caused by the NSP in group 2 may be related to a slower fermentation at a more distal site, resulting in the increase in faecal SCFA concentration. This group had the highest faecal SCFA output. The role of the SCFA in this increase in faecal water is unclear. It was originally thought that SCFA in the colon caused increased stool water by osmotic activity (Forsythe *et al.* 1978). However, it has been shown that SCFA are rapidly absorbed from the colon (McNeil *et al.* 1978) and theoretically should not have a significant osmotic action. In the present study the increased concentrations of faecal SCFA indicate that this absorption capacity is not sufficient to maintain constant concentrations of SCFA and an increased osmotic pressure may still be achieved. The movement of the faecal stream may be related to the balance between production and absorption of SCFA.

When the increase in faecal SCFA concentration is plotted against the SCFA produced by each NSP *in vitro* (Table 1, Adiotomre *et al.* 1990; Edwards & Eastwood, 1992) an n shaped curve is produced (Fig. 1). This supports the hypothesis that fermentability is related to the site of SCFA accumulation, rapidly fermented NSP producing no change in faecal SCFA, less rapidly fermented NSP increasing faecal SCFA, and NSP with low fermentability having little effect on faecal SCFA. Karaya does not fit into this n shaped plot due to the low fermentability *in vitro* compared with an apparent effect on faecal SCFA in the rat *in vivo*. Karaya was anomalous in the predictive index of SCFA stool output in the study of Adiotomre *et al.* (1990) with little effect on stool output in man despite a low fermentability and a high residual WHC. We have since carried out four further *in vitro* fermentations with karaya using different human faecal donors and found very little evidence of fermentation. The fermentability of karaya in the rat may be higher than that measured in human faeces. Moreover, the enzymes necessary for karaya fermentation may need to be induced by prior ingestion of karaya. Karaya data have thus been omitted from Fig. 1.

Vernia *et al.* (1988) showed that in patients with ulcerative colitis, increased faecal lactate was related to an increase in faecal water. However, this was associated with decreased faecal SCFA concentrations. Faecal SCFA and lactate did not play a role in Crohn's

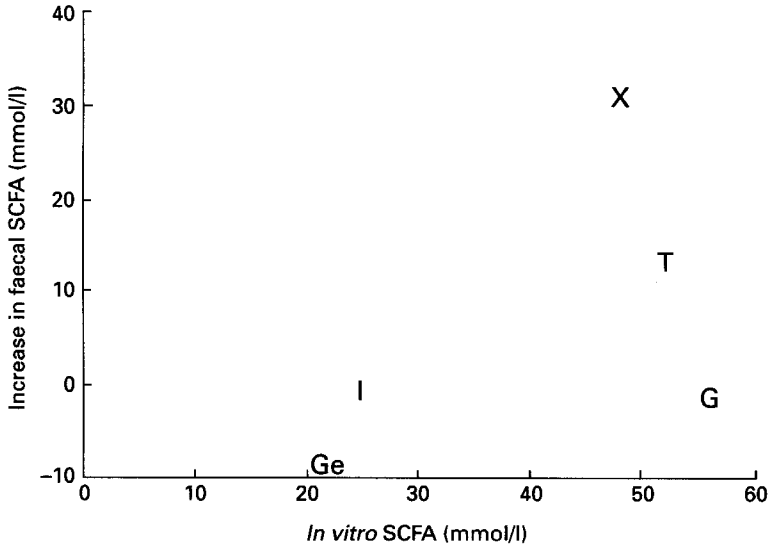

Fig. 1. The relationship between *in vitro* fermentation of non-starch polysaccharides (NSP) measured as total short-chain fatty acids (SCFA) produced, and the increase in faecal SCFA in the rat *in vivo* after 4 weeks ingestion of NSP. G, guar; Ge, gellan; I, ispaghula; X, xanthan; T, tragacanth.

disease. These patients, however, have inflammatory diarrhoea. They have abnormal absorption and motility, as well as probable abnormal carbohydrate fermentation, and their results are not representative of normal colonic physiology. The level of lactate in normal adult faeces is very low. In infants where it is high and in *in vitro* cultures of adult faeces, lactate production is associated with rapid fermentation of sugars or oligosaccharides. Lactate was not measured in the present study but is unlikely to play a role in the fermentation of slowly fermentable NSP. Other studies of ulcerative colitis have shown elevated faecal SCFA (Roediger *et al.* 1982).

There are several other possible mechanisms by which SCFA may affect stool output. SCFA may have significant actions on motility (Yajima, 1985). It is also possible that, due to slow fermentation, the WHC of these NSP is maintained for longer and further round the colon preventing water absorption until the NSP is fermented in a more distal part of the colon with a consequent increase in SCFA. We have previously shown that ispaghula did not increase faecal WHC (Edwards *et al.* 1992). The exact site of the loss of WHC of a NSP may be as important in its action on stool water as a significant increase in the WHC of faeces.

The contribution of bacterial cell mass to the faecal weight was not measured in the present study and it may be possible that there is a different role for the bacterial cells in the action of the different groups of NSP studied (Stephen & Cummings, 1980).

None of the NSP decreased caecal pH despite obvious evidence of fermentation. This is in contrast to previous work in rats (Jacobs & Lupton, 1986; Wyatt *et al.* 1988) and may relate to the lower dose used in the present study. The lower dose may explain why guar gum increased food intake in the present study in contrast to other reports (Wyatt *et al.* 1988). The physical form of the food may also be relevant. The diet in the present study was fed in the form of a paste. The physical form of food is not made clear in other studies. The reason that guar did not increase body weight despite an increased food intake is unclear but may indicate that 2 d measurement of food intake may not fully represent food

intake over the entire 4-week period. There may also be increased energy costs associated with moving viscous NSP along the upper gastrointestinal tract.

Each NSP diet was associated with its own pattern of caecal and faecal SCFA. In the caecum, ispaghula was associated with a higher proportion of propionic acid whereas rats given gellan had more butyric acid. The reason for this difference in end-products is not clear. The predominance of acetic acid is generally higher in the faeces than in the caecum and reflects either a change in fermentation pattern in more distal colon or a difference in the utilization or absorption of the SCFA. However, those fibres which had the greatest effects on faecal water had the least predominance of acetic acid in faeces and in most cases a higher predominance of propionic acid, indicating the possibility of sustained fermentative activity in more distal parts of the colon.

In summary, the rate of fermentation of a dietary fibre and its subsequent effects on caecal and faecal SCFA appears to be an important factor in determining its effect on stool water and stool output. Fibres identified as group 2 in the present study appear to be fermented at a distal site in the colon producing the highest faecal SCFA concentrations and increased stool water. The mechanism for this action is not clear. It may be related to action of the SCFA on motility or a delay in the loss of WHC to a more distal site but the osmotic activity of SCFA produced in the distal colon should not be discounted.

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