Gastrointestinal responses of rats fed on white and wholemeal breads: complex carbohydrate digestibility and the influence of dietary fat content

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To obtain quantitative information on the digestibility of the non-starch polysaccharides (NSP) fraction of white and wholemeal breads, rats were fed on diets in which freeze-dried bread (white, wholemeal or mixtures of the two) provided all the complex carbohydrates. In a second experiment the possibility that dietary fat concentration might influence NSP digestibility was tested by feeding diets containing 30 or 170 g maize oil/kg and either white or wholemeal bread. Multiple linear regression analysis provided little evidence of associative effects of dietary components on NSP digestibility and in the two experiments digestibilities of NSP for white and wholemeal breads were 0·77–0·82 and 0·47–0·52 respectively. Xylose- and arabinose-containing polymers were better digested than was cellulose for both breads. Replacing white by wholemeal bread markedly increased the molar proportion of butyrate in caecal volatile fatty acids at the expense of acetate. This was associated with greater flows of organic matter to the large bowel (LB) and a reduction in caecal transit time (Expt 2). There was little detectable effect of dietary maize oil concentration on NSP digestibility or on LB fermentation. All breads contained some starch resistant to pancreatic α-amylase (*EC* 3.2.1.1) without previous treatment with dimethyl sulphoxide. The digestibility of this starch fraction was not significantly different from 1·0 for all diets except that containing wholemeal bread and the higher maize oil concentration where the apparent digestibility was 0·89.

White and wholemeal bread: Dietary fat: Complex carbohydrate digestibility: Large bowel fermentation: Rat

Classical studies comparing the nutritive values of white and wholemeal breads in man were reported 50 years ago with particular attention to the utilization of minerals (McCance & Widdowson, 1942*a, b*) and the digestibility of major food fractions including so-called 'crude fibre' (Macrae *et al.* 1942). Whilst these studies established that 'crude fibre' was digested to some extent in man, with much greater digestibility for white bread where the crude fibre is mainly 'cell envelopes' compared with wholemeal bread rich in the 'woody fibre in the bran' (Macrae *et al.* 1942), the measurement of this carbohydrate fraction was relatively difficult. Improvements in food carbohydrate chemistry (Southgate, 1969) and, in particular, the direct chemical assay of non-starch polysaccharides (NSP) (Englyst & Cummings, 1984) now make it possible to determine, with confidence, the digestibilities of the NSP fraction in man (Stephen *et al.* 1986) and other animals (Goodlad & Mathers, 1990, 1991).

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The present studies were designed to obtain quantitative information on the digestibility of the NSP fraction and its constituent sugars in white and wholemeal breads using the rat. The breads supplied the only complex carbohydrates in the experimental diets, with mixtures of the breads, in addition to each type separately, being fed to test the hypothesis that there is no interaction between these foods in digestibility of NSP. Since it was anticipated that the supply of additional NSP in wholemeal bread would influence large bowel (LB) metabolism (Cheng *et al.* 1987; Goodlad & Mathers, 1990; Mathers *et al.* 1990), detailed studies of caecal fermentation were carried out.

Whilst conventional rat diets are relatively low in fat, human diets, particularly in affluent Western countries, may provide 40% of dietary energy as fat (Department of Health and Social Security, 1984). To determine whether the concentration of dietary fat influences NSP digestibility or LB fermentation, a second experiment was carried out with diets containing 30 or 170 g maize oil/kg and either white or wholemeal bread. In addition to the measurements made in the first experiment, caecal transit time (TT) and organic matter (OM) fermentation in the LB were measured (in an attempt to explain changes in caecal fermentation) and portal volatile fatty acid (VFA) concentrations were determined as an indication of changes in fermentation endproduct supply to the liver.

Brief accounts of parts of the present study have been presented (Key & Mathers, 1987, 1988).

MATERIALS AND METHODS

Two experiments were carried out using similar animals, diets and experimental procedures.

Animals

For each experiment, twenty-four male Wistar rats were purchased (A. Tuck & Sons, Battlesbridge, Essex), housed in stock cages (four rats per cage) and given *ad lib.* access to a stock diet (41B; Oxoid Ltd, Basingstoke, Hants.) for a pre-experimental period of 7 d. Thereafter, rats were randomly allocated to individual Perspex and stainless steel metabolism cages (Thompson, 1970) which permitted complete separation and collection of urine and faeces. Six animals were allocated at random to each experimental diet.

Diet

Fresh standard sliced bread, white and wholemeal (Robertsons Bakers, Carlisle, Cumbria), was frozen at $-20°$ within 4 h of delivery and subsequently freeze-dried. When dry the bread was ground to pass a 1 mm screen and stored at $-20°$ until incorporated into the diets.

Expt 1. Four diets were formulated (Table 1) in which freeze-dried bread provided 900 g air-dry matter/kg as all white bread (W) changing in equal steps to all wholemeal bread (B). The remainder of the diet was identical and contained no complex carbohydrates. Each rat was offered 20 g air-dry diet at 10.00 hours daily with any uneaten food removed, dried and weighed. Water was available *ad lib.*

Expt 2. This experiment was designed to investigate the effect of dietary fat content in addition to bread type. The two 'low-fat' diets (W and B) were identical with those used in Expt 1 whilst the two 'high-fat' diets (WF and BF) contained 170 g maize oil/kg, largely at the expense of bread (Table 1). To maintain similar intakes of energy and of protein, 20 and 17 g/d of the low- and high-fat diets respectively were offered with the high-fat diets containing a higher concentration of casein and amino acids mix.

Cr_2O_3 (2 g/kg) was added to the diets in Expt 2 as a digesta flow marker to enable estimation of digesta flow rates, especially at the terminal ileum, and of caecal transit times (Goodlad & Mathers, 1990).

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

| | Expt 1 | | | | Expt 2 | | | |
Diet code...	W	WB	BW	B	W	WF	B	BF
White bread*	900	600	300	0	900	750	0	0
Wholemeal bread*	0	300	600	900	0	0	900	750
Maize oil	30	30	30	30	30	170	30	170
Casein + amino acid mix†	30	30	30	30	30	50	30	50
Vitamin + mineral mix‡	40	40	40	40	40	30§	40	30§

* Supplied by Robertsons Bakers, Carlisle, Cumbria. Freeze-dried and ground to pass a 1 mm screen.
† Casein–D-lysine–L-methionine (10:2:1, by wt).
‡ Contained (g/kg premix): CaH_2PO_4 300, KCl 100, $MnSO_4.4H_2O$ 3·5, $FeSO_4.7H_2O$ 1·25, $CuCl_2$ 0·2, KIO_3 0·006, choline chloride 34 and (mg/kg premix) Rovimix AD_3 (Roche) 250, Rovimix E50 (Roche) 1500, menadione 12·5, folic acid 15, calcium pantothenate 110, riboflavin 75, thiamine hydrochloride 55, pyridoxine hydrochloride 180, cyanocobalamin 1 plus sucrose to make 1 kg.
§ Contained approximately 1·75 times concentrations of vitamins and minerals (at the expense of sucrose) to compensate for lower amounts of premix supplied and lower bread intake.

Experimental protocol

After an adaptation period of 7 d, faeces were removed daily, frozen and pooled for two subsequent 7 d balance periods. Urine was collected into vessels containing 5 ml 6 M-H_2SO_4 over the same periods. Animals were weighed at 7 d intervals.

Expt 1. Rats were killed by chloroform overdose. The abdomen was opened by a midline incision and the caecum removed and weighed. Caecal digesta pH was measured immediately and directly using a microelectrode attached to a standard pH meter and samples were taken for VFA and dry matter (DM) determinations. The caecum was gently washed free of digesta using saline (9 g NaCl/l), blotted dry and weighed. Two faecal pellets from the terminal colon were removed for DM determination.

Expt 2. Rats were anaesthetized with diethyl ether, the abdomen was opened and blood removed from the portal vein (1–3 ml) and the heart (2–5 ml). Caecal and colonic samples were taken as described for Expt 1.

Analytical methods

N determinations on diets, faeces and urine were made by a Kjeldahl procedure, Cr_2O_3 and VFA in caecal digesta as described by Mathers *et al.* (1990) and 3-hydroxybutyrate (3OHB) enzymically in deproteinized blood (Lloyd *et al.* 1978). Blood VFA were determined by gas–liquid chromatography as described by Goodlad & Mathers (1990). Dietary and faecal NSP were analysed as described by Englyst & Cummings (1984) and resistant starch (RS) by omitting the dimethyl sulphoxide (DMSO) addition step. The fraction of RS measured in this way is largely retrograded amylose (RS_3; Englyst & Kingman, 1990).

Experimental designs, calculations and statistical analyses

Expt 1. The experiment was designed as a single factor study with four treatments (diets) and six replicates (rats) per diet. Values were examined by one-way analysis of variance and orthogonal polynomials used to describe responses to differences in concentrations of white and wholemeal bread in the diets.

Expt 2. Bread type and dietary fat concentration were considered as separate factors each present at two levels and with six replicates per diet. Values were examined by two-factor analysis of variance.

For both experiments, results are presented as means for each diet with their standard errors based on the between-animals within-diets variations with 20 df.

Separate estimates of apparent digestibility of polysaccharides of white and wholemeal breads were obtained by a multiple linear regression (MLR) technique (Zar, 1974) first outlined by Key & Mathers (1990); a similar approach was adopted by Van Dokkum *et al.* (1983) to obtain estimates of the digestibility of 'fibre' in breads added to a basal diet. In the present study the following MLR model was used:

$$Y = \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_1 X_3,$$

where Y was NSP output in faeces, X_1 and X_2 were intakes of NSP from white and wholemeal breads respectively, X_3 has the value of 0 or 1 when wholemeal bread was absent from or present in the diet respectively, α_1 and α_2 are the coefficients of indigestibility for NSP in white and wholemeal breads respectively, and α_3 is the additional effect of wholemeal bread presence on white-bread NSP digestibility.

Computations were made using the Statgraphics (STSC Inc., Rockville, Maryland, USA) package. Apparent digestibilities were calculated by subtracting the appropriate coefficients of indigestibility from unity.

In Expt 2, caecal transit time was calculated as the amount of Cr_2O_3 recovered in that organ divided by Cr_2O_3 intake rate (Goodlad & Mathers, 1990); for further discussion of methodology, see Mathers & Dawson (1991).

RESULTS

Expt 1

Food intake and growth. There was a significant ($P < 0.05$) linear decline in weight gain with increasing proportion of wholemeal bread in the diet which could, in part, be explained by lower DM intakes particularly for diets WB and BW (Table 2). Of probably greater importance, however, was the strongly linear decrease in digestibility of DM as wholemeal replaced white bread in the diet. Whilst there were no significant ($P > 0.05$) between-diets differences in N retention suggesting that lean tissue gain was unaffected by diet, these estimates were associated with a relatively large SEM (Table 2) so that it should not be concluded unequivocally that diet did not influence N retention. Faecal N output increased linearly ($P < 0.001$) with increasing wholemeal bread in the diet.

Caecal fermentation. Variation in the type of bread in the diet had no significant effects on caecal mass, on caecal tissue weight or on the mass of wet digesta in this organ (Table 3). However, the proportion of DM in caecal digesta increased linearly ($P < 0.001$) as wholemeal bread intake increased at the expense of white. The proportion of DM in distal contents was unaffected by diets with an overall mean of 0.40 (SEM 0.015).

The concentration of total VFA in caecal contents tended to fall as white bread was replaced by wholemeal bread in the diet but the changes were not statistically significant ($P > 0.05$) and there were no detectable accompanying changes in caecal digesta pH (Table 4). However, diet had very marked effects on the molar proportions of individual VFA with increasing wholemeal bread intake associated with strong linear falls in acetate and compensatory increases in butyrate and, to a much lesser extent, isobutyrate.

Digestibility of NSP. The MLR model provided a good description of the relationships between intakes of NSP (and its components) from the breads and faecal outputs, $R^2 > 0.94$ except for mannose (0.76), see Table 5. There was no convincing evidence of an associative effect of the presence of wholemeal bread on the digestibility of NSP in white bread as measured by the parameter α_3 (Table 5), although for some components, e.g. cellulose, α_3 was numerically large and was statistically significant for mannose ($P < 0.01$).

Table 2. *Expt 1. Dry matter (DM) intakes and faecal output, weight gain and aspects of nitrogen metabolism by rats given diets containing various proportions of white and wholemeal breads*

(Means for six rats per diet)

| Wholemeal bread in diet (g/kg)†... | 0 | 300 | 600 | 900 | SE of mean | Statistical significance of dietary effects | | |
Diet code...	W	WB	BW	B		Lin	Quad	Dev
DM intake (g/7 d)	117	108	106	114	2·6	NS	**	NS
Wt gain (g/7 d)	30	26	25	24	1·6	*	NS	NS
Faecal DM output (g/7 d)	5·2	6·8	9·0	11·9	0·29	***	NS	NS
DM indigestibility	0·044	0·063	0·085	0·104	0·0014	***	NS	NS
N intake (g/7 d)	2·44	2·34	2·37	2·69	0·058	**	**	NS
Faecal N output (g/7 d)	0·31	0·36	0·40	0·48	0·020	***	NS	NS
Urinary N output (g/7 d)	1·23	1·17	1·25	1·42	0·074	NS	NS	NS
N retention (g/7 d)	0·89	0·82	0·72	0·79	0·065	NS	NS	NS

NS, not significant; Lin, Quad and Dev, linear, quadratic and deviations from linear and quadratic effects of wholemeal bread content of diet respectively.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of diet composition, see Table 1.

Table 3. *Expt 1. Caecal organ, tissue and digesta masses and proportions of dry matter (DM) in caecal and colonic contents from rats given diets containing various proportions of white and wholemeal breads*

(Means for six rats per diet)

| Wholemeal bread in diet (g/kg)†... | 0 | 300 | 600 | 900 | SE of mean | Statistical significance of dietary effects | | |
Diet code...	W	WB	BW	B		Lin	Quad	Dev
Caecal mass (g)	5·9	6·4	6·2	5·1	0·55	NS	NS	NS
Caecal tissue (g)	1·1	1·2	1·0	1·0	0·06	NS	NS	NS
Caecal digesta wet wt (g)	4·9	5·2	5·2	4·0	0·52	NS	NS	NS
Proportion of DM in caecal digesta	0·15	0·17	0·19	0·20	0·004	***	NS	NS
Proportion of DM in distal colonic contents	0·40	0·41	0·42	0·37	0·030	NS	NS	NS

NS, not significant; Lin, Quad and Dev, linear, quadratic and deviations from linear and quadratic effects of wholemeal bread content of diet respectively.

*** $P < 0.001$.

† For details of diet composition, see Table 1.

Apparent digestibilities of NSP and of its major components (cellulose and the arabinoxylans) were considerably greater for white bread than for wholemeal bread. This was also true for the minor NSP monomers, mannose and galactose, with an apparent digestibility of zero for the latter in wholemeal bread (Table 5).

Table 4. *Expt 1. pH, total volatile fatty acids (VFA) concentrations (mmol/kg caecal contents) and molar proportions (mmol/mol) of individual VFA in caeca of rats given diets containing various proportions of white and wholemeal breads*

(Means for six rats per diet)

	0	300	600	900	SE of mean	Statistical significance of dietary effects		
Wholemeal bread in diet (g/kg)† ...								
Diet code ...	W	WB	BW	B		Lin	Quad	Dev
Caecal pH	6·1	6·2	6·1	6·1	0·10	NS	NS	NS
Total VFA (mmol/kg caecal contents)	131	125	126	120	5·3	NS	NS	NS
Molar proportions of individual VFA (mmol/mol)								
Acetate	659	602	554	526	19·6	***	NS	NS
Propionate	161	171	152	166	6·5	NS	NS	*
Isobutyrate	6	8	7	10	0·9	*	NS	NS
Butyrate	153	196	267	274	20·5	***	NS	NS
Isovalerate	9	11	9	12	1·0	NS	NS	NS
Valerate	12	12	11	12	0·5	NS	NS	NS

NS, not significant; Lin, Quad and Dev, linear, quadratic and deviations from linear and quadratic effects of wholemeal bread content of diet respectively.

* $P < 0.05$, *** $P < 0.001$.

† For details of diet composition, see Table 1.

Table 5. *Expt 1. Apparent digestibilities estimated by multiple linear regression (MLR)* for non-starch polysaccharides (NSP) in white and wholemeal breads when fed in mixed diets to growing rats*

(Mean values with their standard errors; estimates were derived using individual values
for all twenty-four rats)

	White bread		Wholemeal bread		α_3		
	Mean	SE	Mean	SE	Mean	SE	R^2
NSP	0·82	0·057	0·47	0·015	0·08	0·113	0·97
NCP	0·85	0·047	0·52	0·014	−0·13	0·090	0·97
Cellulose	0·64	0·180	0·30	0·024	−0·67	0·351	0·96
Arabinose	0·88	0·054	0·46	0·015	−0·11	0·103	0·97
Xylose	0·90	0·061	0·62	0·016	−0·14	0·116	0·94
Mannose	0·78	0·058	0·58	0·040	−0·30	0·112	0·76
Galactose	0·47	0·045	−0·06	0·032	−0·24	0·089	0·98
Glucose	0·75	0·093	0·30	0·022	−0·28	0·180	0·97

α_3, Additional effect of presence of wholemeal bread on indigestibility of NSP fraction in white bread; NCP, non-cellulosic polysaccharides.

* For details of MLR procedures, see Goodlad & Mathers (1991).

Expt 2

Food intake and growth. The significantly ($P < 0.001$) lower DM intakes of the high-fat diets (WF and BF) when compared with the low-fat diets (W and B) were as expected given the difference in amounts of food offered to the two groups (Table 6). The indigestibility

of DM was significantly greater with the wholemeal-bread diets (B and BF) and was unaffected by dietary maize oil concentration. As in Expt 1, faecal N output was significantly increased in rats consuming wholemeal bread; the reduction with the high-fat diets is largely a consequence of reduced N intake despite the attempt to maintain this across diets by increasing the proportion of casein + amino acids mix in the high-fat diets (Table 1). N retention was significantly higher for the white-bread (W and WF) than for the wholemeal-bread (B and BF) diets.

Caecal fermentation. Rats eating wholemeal-bread-containing diets had significantly heavier caecums containing more digesta and more caecal tissue (Table 7). As in Expt 1, the proportion of DM in caecal digesta of rats consuming white bread was significantly lower than for those consuming wholemeal bread. Whilst there were no significant effects of diet on the proportion of DM in colonic contents (mean 0·40 (SEM 0·013)), there was a tendency for higher values with the high-fat diets. Caecal TT was considerably shorter for wholemeal-bread-containing diets than for white-bread-containing diets (0·69 and 0·49 (SEM 0·036) d respectively) with no effect of dietary fat content (Table 7).

Flow of DM from the terminal ileum was significantly lower for white-bread-based diets than for wholemeal-bread-based diets and for high-fat diets than for low-fat diets (Table 8). To a large extent the latter difference was due to the planned lower DM intakes with the high-fat diets (Table 6) and when expressed in terms of apparent digestibility at the ileum there was no significant dietary fat effect but a highly significant bread effect (Table 8). The latter effect was still clearly apparent in measurements made on caecal digesta and in faeces. Flows of OM into and from the LB followed the same pattern as for DM flows. An estimate of apparently fermented OM was obtained as the difference between ileal supply and faecal OM. When expressed in absolute terms (g/d), apparently fermented OM was significantly lower for animals fed on high-fat diets than for those fed on low-fat diets. However, when expressed as a proportion of ileal OM flow, disappearance of OM in the LB was very significantly higher for rats fed on white bread than for wholemeal bread with no significant effect of dietary fat content.

There were no significant effects of diet on caecal pH or total VFA concentrations (Table 9). However, as in Expt 1, consumption of wholemeal bread was accompanied by large increases in the molar proportion of butyrate and compensatory decreases in acetate and, in addition, propionate (Expt 2 only). Among the minor VFA only valerate was significantly affected by diet, with lower molar proportions with high-fat intakes especially in conjunction with white bread.

Portal blood acetate concentrations appeared highly variable between animals and, although no significant dietary effects were detected, concentrations tended to be higher with diets B and BF (Table 10). Portal propionate concentrations were, on average, 3·2 and butyrate concentrations 4·3 times higher with the diets containing wholemeal bread than with those containing white bread so that total VFA in portal blood was significantly ($P < 0.05$) greater with diets B and BF than with diets W and WF. Acetate was the only VFA detected in peripheral blood, was not affected by diet and its concentration was, on average, only 40% of that in portal blood. Concentrations of 3OHB were very similar in portal and heart blood and, in each case, were significantly ($P < 0.001$) higher with the wholemeal-bread diets. Dietary fat concentration had no detectable effect on any of the measured blood metabolites.

Digestibility of NSP and of RS. Bread type had a marked effect on apparent digestibility of NSP and all its major constituents with very significantly higher values for white-bread-containing diets for NSP, NCP, cellulose, arabinose, xylose and glucose (Table 11). The concentration of maize oil in the diet had no significant effect on any of these components, although there was a significant ($P < 0.001$) interaction between bread type and maize oil

Table 6. *Expt 2. Effects of bread type and dietary fat content on dry matter (DM) intake and faecal output and aspects of nitrogen (N) metabolism in rats*

(Means for six rats per diet)

						Statistical significance of dietary effects		
Maize oil in diet (g/kg)† ...	30	170	30	170	SE of			Inter-
Diet code ...	W	WF	B	BF	mean	Bread	Fat	action
DM intake (g/7 d)	116	105	114	99	3·1	NS	***	NS
Faecal DM output (g/7 d)	5·6	5·4	12·3	10·1	0·36	***	**	*
Apparent DM indigestibility	0·048	0·051	0·107	0·102	0·0027	***	NS	NS
N intake (g/7 d)	2·58	2·33	2·81	2·28	0·068	NS	***	NS
Faecal N output (g/7 d)	0·32	0·26	0·47	0·36	0·018	***	***	NS
Urinary N output (g/7 d)	1·12	0·94	1·35	1·01	0·043	**	***	NS
N retention (g/7 d)	1·14	1·12	1·00	0·91	0·056	**	NS	NS

W, B, white and wholemeal breads respectively; NS, not significant.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of diet composition, see Table 1.

Table 7. *Expt 2. Effects of bread type and dietary fat content on caecal organ, tissue and digesta masses, proportions of dry matter (DM) in caecal and colonic contents and caecal transit time (TT) of rats*

(Means for six rats per diet)

						Statistical significance of dietary effects		
Maize oil in diet (g/kg)† ...	30	170	30	170	SE of			Inter-
Diet code ...	W	WF	B	BF	mean	Bread	Fat	action
Caecal mass (g)	4·1	3·5	4·6	4·5	0·18	***	NS	NS
Caecal tissue (g)	1·0	0·9	1·1	1·1	0·04	*	NS	NS
Caecal digesta wet weight (g)	3·1	2·6	3·6	3·4	0·16	***	*	NS
Proportion of DM in caecal digesta	0·19	0·17	0·21	0·22	0·008	***	NS	NS
Proportion of DM in colonic contents	0·35	0·41	0·38	0·44	0·033	NS	NS	NS
Caecal TT (d)‡	0·76	0·62	0·46	0·52	0·051	***	NS	NS

W, B white and wholemeal breads respectively; NS, not significant.

* $P < 0.05$, *** $P < 0.001$.

† For details of diet composition, see Table 1.

‡ Determined as described by Goodlad & Mathers (1990).

concentration for the minor sugar mannose where digestibility appeared to increase with the higher fat content for white-bread diets with the opposite occurring for wholemeal-bread diets. Digestibility of RS (probably mainly retrograded amylose (RS_3); Englyst & Kingman, 1990) was not significantly different from unity for all diets except the wholemeal-bread diet with the higher maize oil content where RS digestibility was 0·89.

Table 8. *Expt 2. Effects of bread type and dietary fat content on ileal outputs of dry matter (DM) and organic matter (OM), apparent digestibility of DM at different sites in the intestine and OM disappearance in the large bowel (LB) of rats*

(Means for six rats per diet)

						Statistical significance of dietary effects		
Maize oil in diet (g/kg)†...	30	170	30	170	SE of			Inter-
Diet code...	W	WF	B	BF	mean	Bread	Fat	action
Dry matter								
Ileal flow (g/d)	3·7	2·7	4·5	3·7	0·33	*	**	NS
Apparent digestibility								
Ileum	0·77	0·82	0·73	0·73	0·021	**	NS	NS
Caecum	0·95	0·95	0·90	0·90	0·004	***	NS	NS
Faeces	0·96	0·95	0·90	0·91	0·001	***	NS	NS
Organic matter								
Ileal flow (g/d)	2·2	1·5	3·4	2·7	0·21	***	**	NS
Faecal output (g/d)	0·6	0·5	1·6	1·2	0·04	***	***	***
LB disappearance (g/d)	1·6	1·0	1·9	1·5	0·23	NS	*	NS
LB disappearance (g/g ileal OM flow)	0·72	0·62	0·53	0·54	0·034	***	NS	NS

W, B white and wholemeal breads respectively; NS, not significant.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† For details of diet composition, see Table 1.

Table 9. *Expt 2. Effects of bread type and dietary fat content on caecal pH, total volatile fatty acids (VFA) and molar proportions (mmol/mol) of individual VFA in rats*

(Means for six rats per diet)

						Statistical significance of dietary effects		
Maize oil in diet (g/kg)†...	30	170	30	170	SE of			Inter-
Diet code...	W	WF	B	BF	mean	Bread	Fat	action
Caecal pH	6·1	6·2	6·1	6·1	0·10	NS	NS	NS
Total VFA (mmol/kg caecal contents)	156	173	158	166	6·8	NS	NS	NS
Molar proportions of individual VFA (mmol/mol)								
Acetate	739	761	602	627	1·3	***	NS	NS
Propionate	151	150	110	114	0·6	***	NS	NS
Isobutyrate	16	9	11	11	0·2	NS	NS	NS
Butyrate	68	62	252	225	1·4	***	NS	NS
Isovalerate	11	9	12	11	0·1	NS	NS	NS
Valerate	15	9	13	12	0·1	NS	***	*

W, B white and wholemeal breads respectively; NS, not significant.
* $P < 0.05$, *** $P < 0.001$.
† For details of diet composition, see Table 1.

Table 10. *Expt 2. Effects of bread type and dietary fat content on concentrations of volatile fatty acids (VFA; μM) and 3-hydroxybutyrate (3OHB; μM) in blood from the portal vein and heart of rats*

(Means for six rats per diet)

| | | | | | | Statistical significance of dietary effects | | |
| Maize oil in diet (g/kg)†... | 30 | 170 | 30 | 170 | SE of | | | Inter- |
Diet code...	W	WF	B	BF	mean	Bread	Fat	action
Portal blood								
Acetate	606	724	926	1599	304·8	NS	NS	NS
Propionate	108	65	219	338	86·7	*	NS	NS
Butyrate	18	22	111	59	14·6	***	NS	NS
Total VFA	732	811	1256	1996	385·8	*	NS	NS
3OHB	69	59	122	110	5·7	***	NS	NS
Heart blood								
Acetate	404	366	400	372	28·9	NS	NS	NS
3OHB	71	70	122	110	5·5	***	NS	NS

W, B white and wholemeal breads respectively; NS, not significant.
* $P < 0.05$, *** $P < 0.001$.
† For details of diet composition, see Table 1.

Table 11. *Expt 2. Apparent digestibilities of non-starch polysaccharides (NSP) and of resistant starch (RS) for white and wholemeal breads in rats given diets of differing dietary fat content with bread providing the only source of complex carbohydrate in these diets*

(Means for six rats per diet)

| | | | | | | Statistical significance of dietary effects | | |
| Maize oil in diet (g/kg)†... | 30 | 170 | 30 | 170 | SE of | | | Inter- |
Diet code...	W	WF	B	BF	mean	Bread	Fat	action
NSP	0·77	0·81	0·52	0·50	0·018	***	NS	NS
NCP	0·80	0·84	0·60	0·58	0·015	***	NS	NS
Cellulose	0·49	0·48	0·20	0·13	0·043	***	NS	NS
Arabinose	0·85	0·89	0·47	0·48	0·017	***	NS	NS
Xylose	0·87	0·90	0·63	0·63	0·016	***	NS	NS
Mannose	0·52	0·78	0·71	0·59	0·041	NS	NS	***
Galactose	0·27	0·29	0·31	0·35	0·043	NS	NS	NS
Glucose	0·74	0·74	0·41	0·34	0·027	***	NS	NS
RS	0·99	0·99	1·05	0·89	0·027	NS	*	**

W, B white and wholemeal breads respectively; NCP, non-cellulosic polysaccharides; NS, not significant.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† For details of diet composition, see Table 1.

DISCUSSION

Determination of the digestibility of individual foods or ingredients in man presents a major problem in that, with the exception of unweaned infants, mixed diets are habitually eaten and the consumption of only a single food for the time necessary to obtain reliable estimates of digestibility is impractical. One possible solution to this problem is to supply

the test food or ingredient in addition to a basal diet and to attribute any additional faecal material to the indigestible fraction of the test food (van Dokkum *et al.* 1983; Stephen *et al.* 1986). This assumes, implicitly or explicitly, that the addition of the test food has no effect on the digestibility of the basal diet (or vice versa), i.e. that there are no associative effects; an assumption, with uncertain validity, which has long been recognized (see Macrae *et al.* 1942). An alternative approach, used for example by Macrae *et al.* (1942) and McCance & Walsham (1948), is to arrange that the test foodstuff makes up as much as possible of the experimental diets and that the other components (necessary for palatability or to ensure nutritional adequacy) contain a minimum of the nutrients under investigation. For example, in the studies of Macrae *et al.* (1942) bread contributed 74 and 90 % of the energy and N intakes respectively and virtually all the complex carbohydrates, whilst in the studies of McCance & Widdowson (1942 a) bread provided 40–50 % of energy and most of the calcium, the main mineral under investigation. In calculating apparent digestibilities, Macrae *et al.* (1942) attributed all the faecal output to the breads under test whilst McCance & Widdowson (1942 a) and McCance & Walsham (1948) referred to digestibilities of particular diets rather than to the individual breads which were their largest components.

In our experimental diets, bread, either white or wholemeal, provided all the complex carbohydrates and approximately 90 % of DM intake. For Expt 1, where we made a direct comparison of the two types of bread, the other diet ingredients remained constant so that changes in faecal carbohydrate output could be strictly attributed to the breads being investigated. The feeding of such defined and uniform diets to rats over a sufficiently long period can produce both precise and accurate estimates of the digestibility of bread NSP. Whilst such estimates obtained in the rat may also apply to man (Nyman *et al.* 1986), there have been insufficient direct between-species comparisons to permit the unequivocal assumption that results from one species will always apply to the other (for discussion, see Mathers, 1991).

Digestibility of complex carbohydrates. Direct comparison of the results from the present study with those in the literature on the digestibility of complex carbohydrates in breads are complicated, not only by problems of experimental protocol but also by differences in the methods used to determine the non-starch fraction of the polysaccharides. Early studies by, for example, Macrae *et al.* (1942) and McCance & Walsham (1948) used the unphysiological 'crude fibre' method whilst van Dokkum *et al.* (1983) used van Soest's detergent-gravimetric methods (van Soest & Wine, 1967). The most comparable study is that by Ranhotra *et al.* (1988) who, using the 'dietary fibre' method of Prosky *et al.* (1984), obtained digestibilities of 82 and 44 % for white and wholemeal breads respectively in the rat. These are similar to the values for NSP digestibility of 77–82 % for white bread, and 47–52 % for wholemeal bread obtained in our experiment (Tables 5 and 11). Apparent digestibility of NSP in a white-bread-based diet consumed by human volunteers was 78 % with a calculated digestibility of only 33 % (range 0–62 %) for wheat bran added to the basal diet (Stephen *et al.* 1986).

Starch which escapes small intestinal digestion (RS) is subject to fermentation by the LB flora (Macfarlane & Englyst, 1986). Our breads, in agreement with the results of Englyst & Cummings (1984), contained some starch which was resistant to pancreatic α-amylase (*EC* 3.2.1.1) without previous treatment with DMSO and was probably present as RS_3 (Englyst & Kingman, 1990). This fraction disappeared almost entirely during passage through the gut for all diets except the wholemeal-bread diet containing 170 g maize oil/kg (BF; Table 11). The appearance of starch in the faeces of some subjects eating both white and bran-enriched breads was reported by Stephen *et al.* (1986). In an earlier study (Goodlad & Mathers, 1991) we were unable to detect RS in the faeces of rats given raw or cooked peas (*Pisum sativum*) in contrast to the results of Faulks *et al.* (1989). Given the

recent recognition of RS as an important food component (Englyst & Cummings, 1987) and the limited studies on its digestibility, it would be unsafe to assume that all dietary RS is fermented in the LB.

Knowledge of the digestibility of NSP and of RS is of practical value since this is the primary determinant of the energy value of these carbohydrate fractions (Livesey, 1990; Mathers, 1991). Current food tables (Paul & Southgate, 1978), whilst recognizing that NSP may be fermented and produce VFA which are absorbed, discount the energy value of this fraction because of compensatory increases in faecal excretion of fat and protein when diets rich in NSP are consumed (Southgate & Durnin, 1970). However, reappraisal of published results (Livesey, 1990) and the development of models to estimate energy gain from NSP digestion in the LB (e.g. Mathers, 1991) suggest that nutritionally important amounts of energy may be salvaged by LB fermentation. If this is so then quantitative information on the digestibility of NSP, especially for NSP-rich foods, may be of value in compilations of the energy values of foods.

Associative effects on digestibility. When two or more foods or ingredients are consumed together it is possible that the digestibilities of this mixture will not be the weighted sum of the digestibilities of the individual dietary components, i.e. that there may be associative effects. In a recent extensive series of studies with rats fed on mixtures of cereal- and legume-based foods, Hansen *et al.* (1991) reported that whilst, in most cases, digestibility coefficients (DC) for such mixtures can be calculated with high precision for DM, energy, protein, starch and 'dietary fibre' from DC values for individual ingredients, where mixtures contained large amounts of soluble 'dietary fibre' measured DC for the latter fraction may be considerably lower than calculated values. Goodlad & Mathers (1991) found no evidence for associative effects in digestibility of NSP in pigs given wheat-based diets containing 0–300 g raw peas/kg. Similarly, there were no detectable associative effects for NSP digestibility between white and wholemeal breads in the present study. The apparent interaction between bread type and dietary maize oil concentration in digestibility of mannose (Table 11) may be real but is unlikely to have any nutritional significance given the small concentration of mannose in bread NSP. In the absence of anti-nutritional factors influencing host digestive enzyme action or absorption, or of dietary substances affecting the activity of the LB flora, it seems unlikely that there will be important associative effects for common human foods. However, substances which influence TT through the LB may have an impact on the apparent digestibility of NSP (Stephen *et al.* 1987) with consequential effects on the energy yield from this carbohydrate fraction (Mathers, 1991).

LB fermentation. The most striking effect on LB fermentation of replacing white by wholemeal bread was the large increase in molar proportion of butyrate in caecal VFA largely at the expense of acetate (Tables 4 and 9). It is of interest that these changes were linear with respect to changes in proportions of white and wholemeal breads (Table 4). Similar linearity in response of caecal VFA pattern was reported for addition of peas to a basal NSP-free diet by Goodlad & Mathers (1990). We have no satisfactory explanation for the greater increase in butyrate in Expt 2, although it should be noted that it is largely due to a much lower butyrate molar proportion with white bread in Expt 2 when compared with Expt 1. Clearly this change in pattern of fermentation endproducts is associated with a substantial alteration in the chemical composition and amount of substrate delivered to the caecum from the terminal ileum (Table 8) and it might be argued that more NSP *per se* may induce changes in bacterial metabolism. Certainly there is evidence that rat caecal VFA pattern is readily altered by increases in NSP intake with high butyrate proportions reported with cereal sources of NSP (Cheng *et al.* 1987; Mathers *et al.* 1990). However, we have argued elsewhere that substrate supply may not necessarily be the only or indeed the

major influence on fermentation pattern (Goodlad & Mathers, 1990). The proportion of butyrate may be influenced by caecal TT showing sharp increases below TT of 0·6–0·7 d (Mathers & Dawson, 1991). In Expt 2 caecal TT fell from, on average, 0·69 d with the white-bread diets to 0·49 d with the wholemeal-bread diets (Table 7) as would be expected from the greater intake of NSP with the latter diet (van Dokkum et al. 1983; Stephen et al. 1986). The importance or otherwise of caecal TT in determining VFA pattern might be resolved by investigating the effects of pharmacological alteration of TT with no change in NSP intake (see Stephen et al. 1987).

For the rats in Expt 2 the amount of OM fermented in the LB was not affected by bread type but because there was more OM delivered to the LB with the wholemeal-bread diets, the fraction of ileal OM apparently fermented in the LB was significantly lower for the latter diets (Table 8). Therefore, although the wholemeal-bread diets provided much greater intakes of NSP and more OM flowing to the LB than did the white-bread diets, where was no between-diet difference in estimated VFA absorption from the LB (F. B. Key & J. C. Mathers, unpublished results). If all the ingested NSP flowed to the LB (Englyst & Cummings, 1985) then this carbohydrate fraction accounted for 0·15–0·37 of the OM leaving the terminal ileum and 0·16–0·35 of the OM apparently fermented in the LB. Other food components and endogenous materials clearly play a significant part in this aspect of LB metabolism (Cummings & Englyst, 1987).

The relative lack of effect of dietary maize oil on caecal fermentation (including the digestibility of NSP) is probably due to the high digestibility of triacylglycerols in the small bowel so that only small amounts of fat appear in ileal effluent (McNeil et al. 1982) even in subjects with relatively high intakes (Higham & Read, 1990). An intriguing report of reduced methane production with soya-bean oil addition to the diet (independent of changes in non-fat intake) has been reported for the pig by Christensen & Thorbek (1987), whilst Mallett et al. (1983) found that very-high-fat diets (350 g/kg) fed to rats, in general, decreased the activities of caecal microbial enzymes. No mechanisms for these effects have been established and it is possible that the effects are indirect and occur via bacteriostatic effects of additional bile salts flowing to the LB in response to the additional triacylglycerol intake.

Conclusion

There were no important associative effects between type of bread in the diet or dietary maize oil concentration on digestibility of NSP or on caecal fermentation pattern. Increasing NSP intake by replacing white bread with wholemeal bread in the diet of rats provoked large increases in caecal butyrate molar proportion which could not be explained by the amount of OM fermented in the LB.

REFERENCES

Cheng, B.-Q., Trimble, R. P., Illman, R. J., Stone, B. A. & Topping, D. L. (1987). Comparative effects of dietary wheat bran and its morphological components (aleurone and pericarp-seed coat) on volatile fatty acid concentrations in the rat. British Journal of Nutrition 57, 69–76.

Christensen, K. & Thorbek, G. (1987). Methane excretion in the growing pig. British Journal of Nutrition 57, 355–361.

Cummings, J. H. & Englyst, H. N. (1987). Fermentation in the human large intestine and the available substrates. American Journal of Clinical Nutrition 45, 1243–1255.

Department of Health and Social Security (1984). Diet and Cardiovascular Disease. Report of the Committee on Medical Aspects of Food Policy. London: H.M. Stationery Office.

Englyst, H. N. & Cummings, J. H. (1984). Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as the alditol acetates. *Analyst* **109**, 937–942.

Englyst, H. N. & Cummings, J. H. (1985). Digestion of the polysaccharides of some cereal foods in the human small intestine. *American Journal of Clinical Nutrition* **42**, 778–787.

Englyst, H. N. & Cummings, J. H. (1987). Resistant starch, a 'new' food component: a classification of starch for nutritional purposes. In *Cereals in a European Context*, pp. 221–233 [I. D. Morton, editor]. Chichester: Ellis Horwood.

Englyst, H. N. & Kingman, S. M. (1990). Dietary fiber and resistant starch. A nutritional classification of plant polysaccharides. In *Dietary Fiber*, pp. 49–65 [D. Kritchevsky, C. Bonfield and J. W. Anderson, editors]. New York: Plenum Publishing Corporation.

Faulks, R. M., Southon, S. & Livesey, G. (1989). Utilization of α-amylase (*EC* 3.2.1.1)-resistant maize and pea (*Pisum sativum*) starch in the rat. *British Journal of Nutrition* **61**, 291–300.

Goodlad, J. S. & Mathers, J. C. (1990). Large bowel fermentation in rats given diets containing raw peas (*Pisum sativum*). *British Journal of Nutrition* **64**, 569–587.

Goodlad, J. S. & Mathers, J. C. (1991). Digestion by pigs of non-starch polysaccharides in wheat and raw peas (*Pisum sativum*) fed in mixed diets. *British Journal of Nutrition* **65**, 259–270.

Hansen, I., Larsen, T., Bach Knudsen, K. E. & Eggum, B. O. (1991). Nutrient digestibilities in ingredients fed alone or in combinations. *British Journal of Nutrition* **66**, 27–35.

Higham, S. E. & Read, N. W. (1990). Effect of ingestion of fat on ileostomy effluent. *Gut* **31**, 435–438.

Key, F. B. & Mathers, J. C. (1987). Response of rat caecal metabolism to varying proportions of white and wholemeal breads. *Proceedings of the Nutrition Society* **46**, 11A.

Key, F. B. & Mathers, J. C. (1988). Response of rat caecal metabolism to white and wholemeal breads given at two fat levels. *Proceedings of the Nutrition Society* **47**, 101A.

Key, F. B. & Mathers, J. C. (1990). Estimation of the digestibilities of NSP for wholemeal bread and haricot beans fed in mixed diets. In *Dietary Fibre: Chemical and Biological Aspects*, pp. 254–258 [D. A. T. Southgate, K. Waldron, I. T. Johnson and G. R. Fenwick, editors]. Cambridge: Royal Society of Chemistry.

Livesey, G. (1990). Energy values of unavailable carbohydrate and diets: an enquiry and analysis. *American Journal of Clinical Nutrition* **51**, 617–637.

Lloyd, B., Burrin, J., Smythe, P. & Alberti, K. G. M. M. (1978). Enzymic fluorimetric continuous flow assays for blood glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate. *Clinical Chemistry* **34**, 1724–1729.

McCance, R. A. & Walsham, C. M. (1948). The digestibility and absorption of the calories, proteins, purines, fat and calcium in wholemeal wheaten bread. *British Journal of Nutrition* **2**, 26–41.

McCance, R. A. & Widdowson, E. M. (1942*a*). Mineral metabolism of healthy adults on white and brown bread dietaries. *Journal of Physiology* **101**, 44–85.

McCance, R. A. & Widdowson, E. M. (1942*b*). Mineral metabolism on dephytinized bread. *Journal of Physiology* **101**, 304–313.

Macfarlane, G. T. & Englyst, H. N. (1986). Starch utilization by the human large intestinal microflora. *Journal of Applied Bacteriology* **60**, 195–201.

McNeil, N. I., Bingham, S., Cole, T. J., Grant, A. M. & Cummings, J. H. (1982). Diet and health of people with an ileostomy. 2. Ileostomy function and nutritional state. *British Journal of Nutrition* **47**, 407–415.

Macrae, T. F., Hutchinson, J. C. D., Irwin, J. O., Bacon, J. S. D. & McDougall, E. I. (1942). Comparative digestibility of wholemeal and white breads and the effect of the degree of fineness of grinding of the former. *Journal of Hygiene* **42**, 423–435.

Mallett, A. K., Rowland, I. R. & Wise, A. (1983). Influence of dietary fats on rat caecal microflora. *Proceedings of the Nutrition Society* **43**, 7A.

Mathers, J. C. (1991). Digestion of non-starch polysaccharides by non-ruminant omnivores. *Proceedings of the Nutrition Society* **50**, 161–172.

Mathers, J. C. & Dawson, L. D. (1991). Large bowel fermentation in rats eating processed potatoes. *British Journal of Nutrition* **66**, 313–329.

Mathers, J. C, Fernandez, F., Hill, M. J., McCarthy, P. T., Shearer, M. J. & Oxley, A. (1990). Dietary modification of potential vitamin K supply from enteric bacterial menaquinones in rats. *British Journal of Nutrition* **63**, 639–652.

Nyman, M., Asp, N.-G., Cummings, J. & Wiggins, H. (1986). Fermentation of dietary fibre in the intestinal tract: comparison between man and rat. *British Journal of Nutrition* **55**, 487–496.

Paul, A. A. & Southgate, D. A. T. (1978). *McCance and Widdowson's The Composition of Foods*, 4th revised ed. London: H.M. Stationery Office.

Prosky, L., Asp, N. G., Furda, I., Devries, J. W., Schweizer, T. F. & Harland, B. F. (1984). Determination of total dietary fibre in foods, food products and total diets: interlaboratory study. *Journal of the Association of Official Analytical Chemists* **67**, 1044–1051.

Ranhotra, G. S., Gelroth, J. A. & Bright, P. H. (1988). Effect of the source of fiber in bread on intestinal responses and nutrient digestibilities. *Cereal Chemistry* **65**, 9–12.

Southgate, D. A. T. (1969). Determination of carbohydrate in foods. II. Unavailable carbohydrate. *Journal of the Science of Food and Agriculture* **20**, 331–339.

Southgate, D. A. T. & Durnin, J. V. G. A. (1970). Calorie conversion factors. An experimental reassessment of the factors used in the calculation of the energy value of human diets. *British Journal of Nutrition* **24**, 517–521.

Stephen, A. M., Wiggins, H. S. & Cummings, J. H. (1987). Effects of changing transit time on colonic microbial metabolism in man. *Gut* **28**, 601–609.

Stephen, A. M., Wiggins, H. S., Englyst, H. N., Cole, T. J., Wayman, B. J. & Cummings, J. H. (1986). The effect of age, sex and level of intake of dietary fibre from wheat on large-bowel function in thirty healthy subjects. *British Journal of Nutrition* **56**, 349–361.

Thompson, A. (1970). Rat metabolism cage. *Journal of the Institute of Animal Technicians* **21**, 12–21.

Van Dokkum, W., Pikaar, N. A. & Thissen, J. T. N. M. (1983). Physiological effects of fibre-rich types of bread. 2. Dietary fibre from bread: digestibility by the intestinal microflora and water-holding capacity in the colon of human subjects. *British Journal of Nutrition* **50**, 61–74.

Van Soest, P. J. & Wine, R. H. (1967). Use of detergents in the analysis of fibrous feeds. IV. Determination of cell wall constituents. *Journal of the Association of Official Analytical Chemists* **50**, 50–55.

Zar, J. H. (1974). *Biostatistical Analysis*, p. 257. Englewood Cliffs, N.J.: Prentice-Hall.

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