Complex carbohydrate digestion and large bowel fermentation in rats given wholemeal bread and cooked haricot beans (*Phaseolus vulgaris*) fed in mixed diets

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The digestion of non-starch polysaccharides (NSP) and of resistant starch (RS) by rats fed on wholemeal-bread-based diets containing 0–450 g cooked, freeze-dried haricot beans (*Phaseolus vulgaris*)/kg diet was measured over the final 14 d of a 21 d feeding experiment. The bread and beans provided all the dietary polysaccharide. RS could not be detected consistently in faeces and it was assumed that this fraction was entirely fermented in the large bowel (LB). NSP digestibilities were 0·56 and 0·86 for wholemeal bread and beans respectively with no evidence that the dietary presence of beans affected digestibility of bread NSP. Bean non-cellulosic polysaccharides were highly digestible with values of 0·98, 0·88 and 0·99 for arabinose, xylose and uronic acids components respectively. There were large increases in organic matter flow to the LB when beans were fed which was associated with marked caecal hypertrophy and alterations in caecal volatile fatty acids (VFA) pattern. Calculated VFA absorption from the LB was 5-fold higher with the highest level of beans and this was reflected in higher concentrations of VFA in portal and heart blood.

Wholemeal bread: Haricot beans: Complex carbohydrates: Large bowel fermentation: Rat

Mature haricot beans (*Phaseolus vulgaris* seeds) are common human foods. In North America and Western Europe, small-seeded white varieties are processed commercially and consumed as canned baked beans (Tobin & Carpenter, 1978) with average UK consumption being 18 g/head per d (National Food Survey Committee, 1990). Haricot beans are low in fat (approximately 18 g/kg dry matter (DM)) but rich in complex carbohydrates (Paul & Southgate, 1978; Tobin & Carpenter, 1978; Reddy *et al.* 1984), appear to have hypocholesterolaemic effects in man (Shutler *et al.* 1987*a*, *b*, 1989) and are, therefore, a potentially valuable component in the UK diet (Department of Health and Social Security, 1984). Beans are frequently eaten together with cereals and, whilst the protein quality of such mixtures has been studied intensively (for review, see Tobin & Carpenter, 1978), there is little information on the digestion of their complex carbohydrates despite the fact that this fraction accounts for approximately 0·77 and 0·85 of the dry matter (DM) in beans and cereals respectively (Paul & Southgate, 1978). It is now well established that isolated complex carbohydrates (Englyst *et al.* 1987; Tulung *et al.* 1987) or foods rich in non-starch polysaccharides (NSP) (Cheng *et al.* 1987; Goodlad & Mathers 1990; Mathers *et al.* 1990) have marked effects on rat large bowel (LB) fermentation, but there is little information on the effects of mixed diets which are more relevant to man.

The present experiment was designed to quantify the digestion by rats of complex

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carbohydrates, in particular starch resistant to α-amylase (*EC* 3.2.1.1) (resistant starch; RS) and the NSP fraction in mixed diets containing wholemeal bread and cooked haricot beans. Multiple linear regression (MLR) techniques were used to obtain separate estimates of digestibility for wholemeal bread and for beans and also to test the hypothesis that the dietary presence of haricot beans altered the digestibility of bread NSP. The proportion of wholemeal bread in the diet was held constant whilst the contribution of beans increased from 0 to 450 g/kg to enable investigation of the capacity of the rat LB to ferment the increased amount of substrate supplied to that organ. Measurements were also made of the amount of organic matter (OM) fermented in the LB and of the caecal volatile fatty acid (VFA) pattern from which estimates of VFA absorption were derived.

Brief accounts of parts of the present study have been presented (Key & Mathers, 1989, 1990).

MATERIALS AND METHODS

Animals and housing

Twenty-four male Wistar rats were purchased (A. Tuck & Sons, Battlesbridge, Essex) and housed in individual Perspex and stainless steel metabolism cages (Thompson, 1970) which permitted complete separation and collection of urine and faeces.

Diets and feeding

Four diets were formulated (Table 1) each containing an equal concentration of wholemeal bread (500 g/kg air-dry matter) and with graded concentrations of cooked haricot beans. The bread was purchased as 800 g sliced loaves from Robertsons Bakers, Carlisle, Cumbria, cut into approximately 20 mm squares, frozen at $-20°$ within 4 h of delivery and then freeze-dried. The haricot beans were cooked in an autoclave (Astell Heason 2000 series) set at 10 psi for 10 min which gave a total cooking time at $> 100°$ of approximately 30 min. The beans were drained, frozen at $-20°$ and freeze-dried. Both bread and beans were milled to pass a 1 mm screen and stored at $-20°$ until incorporated into the diets. The diets were designed to be isonitrogenous based on the Paul & Southgate (1978) food tables. All diets contained 2 g Cr_2O_3/kg as an indigestible marker to enable estimation of digesta flow rates, especially at the terminal ileum, and of caecal transit times (TT) (Goodlad & Mathers, 1990).

Animals were offered 20 g air-dry diet at 10.00 hours daily with uneaten food removed at the same time the following morning. Water was available *ad lib.*

Experimental protocol

The rats, initial weight 249 (SE 2·6) g, were weighed every 7 d. After 7 d adaptation to the diets there followed two consecutive 7 d balance periods with total collection of faeces and urine and measurement of intake. At the end of this period the animals were injected intraperitoneally with vincristine sulphate (1 mg/kg body weight provided in sterile saline (9 g NaCl/l) containing 0·5 mg vincristine sulphate/ml). After 2 h each animal was anaesthetized with diethyl ether, and blood, digesta and tissue samples collected as described by Goodlad & Mathers (1990). In addition, samples of duodenal, caecal and colonic tissue were taken for histological and enzymological measurements which will be reported separately.

Analytical methods

NSP and its constituents were determined as described by Englyst & Cummings (1984) and RS by omitting the dimethyl sulphoxide (DMSO) addition step. This fraction is probably mainly retrograded amylose (RS_3; Englyst & Kingman, 1990) and may not represent all the

Table 1. *Formulation (g/kg) and analysed composition (g/kg dry matter (DM)) of diets*

Diet...	BH1	BH2	BH3	BH4
Wholemeal bread*	500	500	500	500
Haricot beans† (*Phaseolus vulgaris*)	0	150	300	450
Sucrose	348	233	117	0
Casein + methionine‡	99	66	33	2§
Vitamin and mineral mix‖	26	26	26	26
Maize oil	27	25	24	22
Analysed composition (g/kg DM)				
Nitrogen	26·4	26·4	29·3	29·6
Total NSP	33·1	59·9	75·8	107·5
NCP	27·9	51·8	68·9	96·6
Cellulose	5·3	8·1	6·9	10·9
Arabinose	8·9	16·3	23·3	28·8
Xylose	12·0	15·4	15·3	21·9
Mannose	0·3	0·7	0·5	1·5
Galactose	0·8	2·4	3·0	5·5
Glucose	9·8	19·9	25·3	38·2
Uronic acids	1·4	5·2	8·4	11·6
Resistant starch	3·3	9·2	12·8	17·8

NSP, non-starch polysaccharides; NCP, non-cellulosic polysaccharides; DM, dry matter.

* Supplied by Robertsons Bakers, Carlisle, Cumbria. Freeze-dried and ground to pass a 1 mm screen.

† Autoclaved at 10 psi for 10 min. Total cooking time approximately 30 min. Freeze-dried and ground to pass 1 mm screen.

‡ Casein–L-methionine (10:5, w/w).

§ L-methionine only.

‖ Contained (g/kg premix): CaH_2PO_4 659, KCl 131, $MnSO_4.4H_2O$ 5·4, $FeSO_4.7H_2O$ 6·7, $ZnCl_2.7H_2O$ 2, choline chloride 52, and (mg/kg premix): $CuCl_2.2H_2O$ 310, KIO_3 10, Rovimix AD_3 (Roche) 385, Rovimix E50 (Roche) 2310, menadione 19, folic acid 32, calcium pantothenate 127, riboflavin 96, thiamin hydrochloride 85, niacin 231, cyanocobalamin 1·9 plus sucrose to make 1 kg.

RS in the foods. Dietary, faecal and urinary N were measured by a Kjeldahl procedure, Cr_2O_3 and VFA in caecal digesta as described by Mathers *et al.* (1990), OM by heating at 500° for 16 h, and 3-hydroxybutyrate (3OHB) in deproteinized blood enzymically (Lloyd *et al.* 1978). Concentrations of VFA in portal and heart blood were determined as described by Goodlad & Mathers (1990).

Experimental design, calculations and statistical analysis

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 were used to describe responses to the inclusion of beans in the diets. Results are presented as means for each diet with their standard errors based on between-animals within-diets variation with 20 df. Separate estimates of apparent digestibility of polysaccharides of wholemeal bread and of haricot beans were obtained by an MLR technique (Zar, 1974) first outlined by Key & Mathers (1990). In the present study, two MLR models were used:

$$\text{MLR model 1: } Y = \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_1 X_3,$$

where Y was output of NSP in the faeces, X_1 and X_2 were intakes of NSP from wholemeal bread and beans respectively, X_3 has the value of 0 or 1 when beans were absent from or present in the diet respectively, α_1 and α_2 are the coefficients of indigestibilities for NSP in bread and beans respectively and α_3 is the additional effect of presence of beans on bread NSP indigestibility. Where α_3 is not significant, a simpler model is appropriate:

$$\text{MLR model 2: } Y = \beta_1 X_1 + \beta_2 X_2,$$

where β_1 and β_2 are the coefficients of indigestibility for bread and beans respectively. Computations were carried out using the Statgraphics package (STSC Inc., Rockville, Maryland, USA). Apparent digestibilities were calculated by subtracting the appropriate coefficients of indigestibility from unity.

Digesta flow rates and caecal TT were estimated by classical marker-ratio techniques (Faichney, 1975) as described by Goodlad & Mathers (1990); for further discussion of the robustness of this methodology, see Mathers & Dawson (1991). The rate of flow of a digesta component past a given point in the intestine was calculated as the rate of intake of marker (Cr_2O_3) divided by marker: component in digesta collected from that sampling point. Caecal TT was calculated as the amount of Cr_2O_3 recovered in that organ divided by Cr_2O_3 intake rate.

RESULTS

Diet composition

Including haricot beans in the diet at the expense of sucrose and casein resulted in substantial increases in dietary NSP concentration, largely as non-cellulosic polysaccharides (NCP) and, in particular, polymers containing arabinose, xylose, glucose and uronic acids. Cellulose contributed 0·16 of the NSP in the basal (without beans) diet and 0·10 in the diet containing the highest level of beans (BH4). Mannose and galactose were minor contributors in all diets. There was also a 5-fold increase in RS; probably mainly RS_3.

Food intake and growth

Over the 2 weeks of the balance period there was a small but significant increase in DM intake (mean intakes (g/7 d) were 121 and 127 (SE 1·1) for weeks 1 and 2 respectively) but no significant differences between diets (Table 2). The rats grew equally well on all diets. N intakes were higher with the diets containing higher levels of beans (BH3 and BH4) because of the higher N contents of these diets (Table 1). Faecal N output increased linearly with each increment of beans in the diet and was almost twice as great with diet BH4 (450 g beans/kg diet) than with the basal (without beans) diet. N retention was fairly similar for all diets.

Gastrointestinal measurements

Small intestine (SI) length tended to increase with increasing proportion of haricot beans in the diet but the effect was not statistically significant (Table 3). Adding beans to the diet resulted in heavier caecums with significantly greater weights of both tissue and digesta contents. Both caecal and colonic digesta contained significantly higher proportions of water (less DM per unit digesta) with the diets containing higher levels of beans. Caecal TT was not significantly affected by diet with a mean TT of 0·51 (SEM 0·04) d (Table 3), although TT for the diets containing beans (mean 0·47 d) tended to be lower than that (0·62 d) for the basal diet.

Each addition of beans to the diet was accompanied by very highly significant ($P <$ 0·001) linear increases in the amounts of DM and OM flowing from the terminal ileum to the caecum (Table 4). There were corresponding, but much smaller, increases in faecal outputs of DM and OM so that the amounts of DM and OM disappearing within the LB increased markedly with increasing proportion of beans in the diet. The amount of OM apparently fermented in the LB increased 5-fold. The proportion of ileal OM flow which

Table 2. *Dry matter (DM) intake, growth rate, food conversion ratio (FCR; g food/g gain) and aspects of nitrogen metabolism in rats given wholemeal bread-based diets containing graded concentrations of cooked haricot beans* (Phaseolus vulgaris)

(Means for six rats per diet; each value is based on two consecutive 7 d balance periods)

Diet† ... Bean content of diet (g/kg) ...	BH1 0	BH2 150	BH3 300	BH4 450	SEM	Statistical significance of dietary effects: Lin	Quad	Dev
DM intake (g/7 d)	124	124	127	121	3·0	NS	NS	NS
Growth rate (g/7 d)	32	34	33	31	2·5	NS	NS	NS
FCR	4·0	3·8	4·2	4·1	0·30	NS	NS	NS
N intake (g/7 d)	3·28	3·26	3·73	3·59	0·083	**	NS	**
Faecal N output (g/7 d)	0·44	0·53	0·71	0·84	0·086	***	NS	NS
Urinary N output (g/7 d)	1·53	1·60	1·71	1·56	0·063	NS	NS	NS
N retention (g/7 d)	1·31	1·13	1·31	1·20	0·064	NS	NS	*

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

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† For details of diet composition, see Table 1.

Table 3. *Small intestine (SI) length, caecal tissue and contents weights and transit time and proportion of dry matter (DM) in colonic digesta in rats given wholemeal bread-based diets containing graded concentrations of cooked haricot beans* (Phaseolus vulgaris)

(Means for six rats per diet)

Diet† ... Bean content of diet (g/kg) ...	BH1 0	BH2 150	BH3 300	BH4 450	SEM	Statistical significance of dietary effects: Lin	Quad	Dev
SI length (mm)	1190	1230	1200	1270	21	NS	NS	NS
Caecum								
Organ mass (g)	5·10	5·13	8·63	9·67	0·47	***	NS	*
Tissue (g)	1·04	1·14	1·57	1·85	0·056	***	NS	NS
Wet contents (g)	4·06	3·99	7·07	7·82	0·44	***	NS	NS
Digesta DM (g/g wet digesta)	0·19	0·18	0·17	0·16	0·004	***	NS	NS
Transit time (d)	0·62	0·43	0·50	0·48	0·08	NS	NS	NS
Proportion of DM in colonic contents	0·42	0·42	0·35	0·28	0·029	***	NS	NS

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

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† For details of diet composition, see Table 1.

was apparently fermented in the LB increased curvilinearly from 0·3 to 0·6 as beans were added to the diet (Table 4). Bean consumption resulted in a small but significant ($P < 0·01$) linear decrease in the pH of the caecal contents which did not match changes in caecal total VFA concentrations. However, feeding beans was associated with altered VFA pattern, i.e. higher molar proportions of propionate but less isobutyrate and isovalerate. VFA absorption from the LB estimated from ileal to faecal OM disappearance and caecal VFA

Table 4. *Flows of dry matter and organic matter (OM) to, and disappearance in, the large bowel (LB) of rats given wholemeal bread-based diets containing graded concentrations of cooked haricot beans* (Phaseolus vulgaris)

(Means for six rats per diet)

Diet† ...	BH1	BH2	BH3	BH4		Statistical significance of dietary effects		
Bean content of diet (g/kg)...	0	150	300	450	SEM	Lin	Quad	Dev
Dry matter								
Ileal flow (g/d)	2·5	3·9	5·4	6·3	0·22	***	NS	NS
Faecal output (g/d)	1·4	1·6	2·0	2·4	0·06	***	NS	NS
LB disappearance (g/d)	1·2	2·2	3·3	3·9	0·20	***	NS	NS
Organic matter								
Ileal flow (g/d)	1·6	2·6	3·6	4·5	0·14	***	NS	NS
Faecal output (g/d)	1·1	1·3	1·7	1·9	0·05	***	NS	NS
LB disappearance (g/d)	0·5	1·2	1·9	2·6	0·13	***	NS	NS
LB disappearance (g/g ileal OM flow)	0·3	0·5	0·5	0·6	0·02	***	**	NS

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

** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

† For details of diet composition, see Table 1.

molar proportions showed very highly significant linear increases over a 5-fold range as bean consumption increased (Table 5).

VFA and 3OHB in portal and heart blood

Whilst portal blood acetate concentration tended to increase with increased dietary inclusion of beans the increase was not statistically significant (Table 6). Linear increases in propionate, butyrate and total VFA were, however, highly significant. In heart blood there were also significant linear increases in acetate ($P < 0{\cdot}05$) and propionate ($P < 0{\cdot}001$) concentrations, the latter increasing from undetectable levels with the basal diet (without beans) to 24 μM (diet BH4). With all diets propionate contributed less than 2% of the VFA detected in blood drawn from the heart. 3OHB was always higher in portal than in heart blood but was not significantly affected by diet.

Digestibility of complex carbohydrates

Although including beans in the diet in place of sucrose and casein resulted in increases in dietary NSP concentration of up to 3-fold (Table 1), there were much smaller increases (less than 2-fold) in faecal NSP output (Table 7). However, with the exception of arabinose, where no significant dietary effect was detected, faecal output of all NSP components increased strongly linearly. The measurement of RS in faeces was associated with a large coefficient of variation and faecal RS output was not significantly different from zero for all diets.

The use of MLR procedures allowed the calculation of separate estimates of apparent digestibility for NSP of wholemeal bread and cooked haricot beans and also tested the possibility that the presence of beans might influence the apparent digestibility of bread NSP with the extent of this influence estimated as the parameter, α_3. MLR analysis (model 1) showed that the presence of beans had little effect on the digestibility of NSP or any of

Table 5. *Caecal pH, total volatile fatty acid (VFA) concentrations, molar proportions of individual VFA and calculated absorption of VFA from the large bowel (LB) of rats given wholemeal bread-based diets containing graded concentrations of cooked haricot beans* (Phaseolus vulgaris)

(Means for six rats per diet)

Diet†...	BH1	BH2	BH3	BH4		Statistical significance of dietary effects		
Bean content of diet (g/kg)...	0	150	300	450	SEM	Lin	Quad	Dev
pH	6·2	6·2	6·0	5·9	0·08	**	NS	NS
Total VFA (mmol/kg caecal contents)	185	168	205	194	10·3	NS	NS	*
Molar proportions of individual VFA (mmol/mol)								
Acetate	659	635	652	658	9·8	NS	NS	NS
Propionate	209	256	233	251	8·5	*	NS	**
Isobutyrate	8	4	1	1	0·8	***	*	NS
Butyrate	98	81	92	70	7·9	NS	NS	NS
Isovalerate	12	9	6	6	1·4	***	NS	NS
Valerate	14	15	16	14	1·0	NS	NS	NS
Calculated absorption of VFA from the LB (mmol/d)‡								
Acetate	3·8	9·1	14·7	19·8	1·01	***	NS	NS
Propionate	1·2	3·7	5·2	7·5	0·39	***	NS	NS
Butyrate	0·5	1·2	2·0	2·1	0·80	***	NS	NS
Total	5·5	14·0	21·9	29·4	1·45	***	NS	NS

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

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† For details of diet composition, see Table 1.

‡ Calculated from organic matter disappearance in the large bowel (Table 4) and caecal VFA molar proportions assuming conventional anaerobic stoichiometry (Demeyer & Van Nevel, 1975).

its components in bread (α_3, not significant; Table 8) so that the simpler model 2 could provide satisfactory estimates of digestibility (R^2 0·86–0·99). Apparent digestibility of total NSP of wholemeal bread was 0·56 with apparent digestibilities of individual monomeric constituents ranging from −0·04 (galactose) to 0·99 (uronic acids). NSP of haricot beans were much more digestible (0·86) with individual sugar constituent digestibilities ranging from 0·74 (glucose) to 0·99 (uronic acids). The apparent digestibility of cellulose was relatively low and similar for both bread (0·22) and haricot beans (0·23).

DISCUSSION

Dietary complex carbohydrates

Haricot beans are rich in complex carbohydrates and their inclusion in the diets varied total NSP content over a 3-fold range. In agreement with Englyst & Cummings (1984), arabinose and glucose, the latter largely in NCP, were the major monosaccharide constituents of NSP in these beans. However, the measured concentration of RS (67 g/kg DM) in our beans was approximately nine times that reported by Englyst & Cummings (1984). This raised RS content is probably mainly in the form of RS_3 arising as a consequence of cooking and

Table 6. *Concentrations of volatile fatty acids (VFA; μM) and of 3-hydroxybutyrate (3OHB; μM) in whole blood from the portal vein and heart of rats given wholemeal bread-based diets containing graded concentrations of cooked haricot beans* (Phaseolus vulgaris)

(Means for six rats per diet except where indicated)

Diet†...	BH1	BH2	BH3	BH4		Statistical significance of dietary effects		
Bean content of diet (g/kg)...	0	150	300	450	SEM	Lin	Quad	Dev
Portal blood								
Acetate	1257	1316	1290	1642	158·2	NS	NS	NS
Propionate	55	117	226	256	40·5	***	NS	NS
Butyrate	22	28	57	78	9·6	***	NS	NS
Total	1334‡	1461‡	1573‡	1976§	136·8‡	**	NS	NS
3OHB	141‡	101‡	143	102‖	31·9‡	NS	NS	NS
Heart blood								
Acetate	1220	1022	1318	1970	269·8	*	NS	NS
Propionate	0	7	20	24	5·6	**	NS	NS
3OHB	79	73	87	89	18·6	NS	NS	NS

Lin, Quad, Dev, linear, quadratic and deviations from linear and quadratic effects of bean content of diet respectively; NS, not significant.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.
† For details of diet composition, see Table 1.
‡ *n* 5.
§ *n* 3.
‖ *n* 4.

Table 7. *Faecal outputs (mg/7 d) of non-starch polysaccharides (NSP) and of resistant starch by rats given wholemeal bread-based diets containing graded concentrations of cooked haricot beans* (Phaseolus vulgaris)

(Means for six rats per diet except where indicated)

Diet†...	BH1	BH2	BH3	BH4		Statistical significance of dietary effects		
Bean content of diet (g/kg)...	0	150	300‡	450‡	SEM	Lin	Quad	Dev
NSP	295	366	433	510	20·3	***	NS	NS
NCP	205	229	290	311	14·1	***	NS	NS
Cellulose	90	137	143	199	7·8	***	NS	*
Arabinose	80	79	87	85	4·9	NS	NS	NS
Xylose	78	85	105	105	6·7	**	NS	NS
Mannose	3	3	4	7	0·7	***	NS	NS
Galactose	14	26	27	37	3·7	***	NS	NS
Glucose	120	172	209	274	9·9	***	NS	NS
Uronic acids	< 1	1	1	3	0·2	***	NS	NS
Resistant starch	< 1	18	5	13	8·9	NS	NS	NS

Lin, Quad, Dev, linear, quadratic and deviations from linear and quadratic effects of bean content of diet respectively; NS, not significant; NCP, non-cellulosic polysaccharides.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.
† For details of diet composition, see Table 1.
‡ *n* 5.

Table 8. *Apparent digestibilities, estimated by multiple linear regression (MLR)*, for non-starch polysaccharides (NSP) of wholemeal bread and cooked haricot beans* (Phaseolus vulgaris) *when fed in mixed diets to rats*

(Estimates were derived using individual values for twenty-two rats†; values are means with their standard errors)

	Wholemeal bread		Beans		α_3		
	Mean	SE	Mean	SE	Mean	SE	R^2
MLR model 1							
NSP	0·56	0·028	0·86	0·027	0·00	0·052	0·99
NCP	0·64	0·024	0·92	0·025	−0·01	0·045	0·98
Cellulose	0·16	0·072	0·25	0·054	−0·07	0·085	0·98
Arabinose	0·56	0·026	0·98	0·025	−0·01	0·051	0·98
Xylose	0·68	0·028	0·92	0·063	0·04	0·045	0·97
Mannose	0·55	0·126	0·86	0·049	0·03	0·180	0·85
Galactose	0·08	0·228	0·83	0·074	0·37	0·393	0·91
Glucose	0·40	0·049	0·75	0·036	0·01	0·088	0·99
Uronic acids	0·98	0·009	0·99	0·002	−0·02	0·018	0·89
MLR model 2							
NSP	0·56	0·023	0·86	0·017			0·99
NCP	0·64	0·020	0·92	0·013			0·99
Cellulose	0·22	0·038	0·23	0·045			0·98
Arabinose	0·56	0·022	0·98	0·015			0·98
Xylose	0·67	0·022	0·88	0·046			0·97
Mannose	0·53	0·088	0·85	0·039			0·86
Galactose	−0·04	0·185	0·78	0·050			0·91
Glucose	0·40	0·040	0·74	0·023			0·99
Uronic acids	0·99	0·008	0·99	0·001			0·89

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

* For details of MLR models 1 and 2, see pp. 499–500.

‡ Samples for one rat on each of diets BH3 and BH4 were missing.

cooling the beans since similar procedures have been reported to increase the concentration of RS in sorghum (*Sorghum bicolor* (L.) Moench; Bach Knudsen *et al.* 1988) and in peas (*Pisum sativum*; Goodlad & Mathers, 1992). It should not be assumed that the RS fraction we have measured represents, necessarily, all the starch which will escape digestion in the small intestine. From *in vitro* studies Englyst & Kingman (1990) reported that there was approximately twice as much physically inaccessible starch (RS_1 cf RS_3) in cooked, cooled beans.

Digestibility

The increased faecal DM output accompanying increased dietary bean concentration (Table 4) was, in part, due to greater outputs of NSP (Table 7), but faecal NSP concentration was very similar for all diets (211, 222, 215 and 214 (SE 9·4) g/kg DM for diets BH1, BH2, BH3 and BH4 respectively) so that other components including bacterial biomass, undigested protein from endogenous and dietary sources, and mucins are likely to be important components of this non-NSP faecal DM. The strong linear decline in apparent digestibility of N with increasing bean intake (Table 2) is in accord with many reports of studies in rats, pigs and man (for review, see Tobin & Carpenter, 1978). The reasons for the low protein digestibility in beans include (1) the presence of protease inhibitors (Liener & Kakade, 1980) which can largely be overcome by thermal processing,

(2) protein fractions which are inherently resistant to proteolytic enzymes (Tobin & Carpenter, 1978), (3) enhanced loss of endogenous N, possibly through stimulation of mucosal cell turnover (Skurpakkar *et al.* 1979) although this is disputed by Fairweather-Tait *et al.* (1983), and (4) increased output of bacterial N (Mason & Palmer, 1973; Bender & Mohammidiha, 1981) as a result of LB bacterial proliferation on the additional carbohydrate supplied to that organ by the beans. In these animals we found no evidence that feeding beans altered gut epithelial proliferation rate (Key & Mathers, 1989) and suggest that the major reason for the increased faecal N loss was greater production of bacterial N within the LB (Goodlad & Mathers, 1990, 1991).

MLR procedures were used to obtain separate estimates of apparent digestibility for NSP of wholemeal bread and haricot beans whilst MLR model 1 also tested for associative effects (Mitchell, 1964), i.e. the possibility that the dietary presence of beans affected the digestibility of bread NSP. There was little evidence of the latter (Table 8) as was also reported by Goodlad & Mathers (1991) for mixtures of wheat and raw peas fed to pigs. The apparent digestibility of wholemeal bread NSP (0·56) was a little higher than those (0·47 and 0·53) reported by Key & Mathers (1993) for rats fed on bread from the same bakery and using the same analytical methods. For comparison, Ranhotra *et al.* (1988), using the enzymic–gravimetric method of Prosky *et al.* (1984), reported wholemeal bread NSP digestibility by rats was 0·44 whilst in man apparent digestibility of fibre in wheat-bran-enriched breads were 0·44 (Van Dokkum *et al.* 1983, using the van Soest & Wine (1967) method) and 0·33 (Stephen *et al.* 1986, determined as NSP by essentially the same method as that used in the present study). Pigs digested 0·65 of the NSP in raw wheat (Goodlad & Mathers, 1991). The NSP of haricot beans were much more digestible (0·86) and similar to values for raw and cooked peas (Goodlad & Mathers, 1990, 1992) and for white bread (Key & Mathers, 1993) fed to rats. For both bread and beans cellulose was much less digestible than the NCP, again in agreement with many studies in rats (Goodlad & Mathers, 1990, 1992; Key & Mathers, 1993), man (Southgate *et al.* 1976; Van Dokkum *et al.* 1983) and pigs (Goodlad & Mathers, 1991). Arabinose-containing polymers in beans as in peas (Goodlad & Mathers, 1990, 1991, 1992) were very highly digested, possibly because of their presence in pectin-like water-soluble branched arabinans (Wilder & Albersheim, 1973). The other major NCP monomer, xylose, occurs in haricot beans mainly in the form of xyloglucans which appear to link cellulose fibrils with the rest of the cell wall (Wilder & Albersheim, 1973). This close physical association with the relatively indigestible cellulose may contribute to the lower digestibility of xylose. The RS fraction measured in the diets (probably largely RS_3) could not be detected consistently in faeces and is assumed to have been fermented in the LB (Macfarlane & Englyst, 1986) as observed in earlier studies (Goodlad & Mathers, 1990, 1991, 1992; Key & Mathers, 1993).

LB fermentation

Whilst feeding beans tended to increase the length of the SI (Table 3), the effect was not statistically significant ($P > 0.05$) and the most noticeable effects were in the LB. As expected, there was a large linear increase from 1·6 to 4·5 g/d in the flow of OM to the LB with increasing bean intake. The composition of ileal OM was not determined but the contribution from NSP and measured RS (assuming that these are not digested in the upper tract; Englyst & Cummings, 1985) was approximately 0·7, 1·2, 1·6 and 2·3 g/d for diets BH1, BH2, BH3 and BH4 respectively. This indicates that other sources including oligosaccharides of the raffinose family (Reddy *et al.* 1984), starches (especially RS_1) and proteins of dietary origin and endogenous materials (Cummings & Englyst, 1987) were equally important as LB substrates. The additional OM entering the LB with the diets containing beans was much more fermentable than that flowing with the wholemeal bread only diet (BH1), so that whilst potential substrate supply increased 2·8 times the amount of OM apparently fermented increased 5-fold from 0·5 to 2·6 g/d. The more highly-digestible NSP and the greater RS supply (assumed to be virtually all fermented) will have contributed to this greater OM disappearance within the LB. This greater fermentative activity was associated with a reduction in caecal pH in the absence of a consistent increase in total VFA concentration which suggests that either the buffering capacity of caecal contents was reduced by feeding beans or that some other acidic fermentation endproduct, e.g. lactate, not measured in the present study, was produced. The molar proportions of acetate and butyrate were little affected by diet but propionate was higher with the diets containing beans. The proportion of butyrate with the basal diet (BH1) was considerably lower than that observed in earlier bread-feeding experiments (Key & Mathers, 1993) and did not increase with increasing intake of complex carbohydrates. We have argued (Mathers & Dawson, 1991) that whilst substrate supply can have marked effects on the pattern of fermentation endproducts (Englyst *et al.* 1987; Goodlad & Mathers, 1988) other environmental factors including pH and TT are also important, and that reduction in caecal TT may be particularly important in increasing the proportion of butyrate. In support of this suggestion there is the observation that diet had no significant effect on caecal TT in the present study. The highly significant reductions in isobutyrate and isovalerate indicate that the balance between their production from amino acid catabolism and their use for *de novo* synthesis (Russell & Hespell, 1981) shifted towards the latter in animals fed on beans.

The greater OM fermentation with the diets containing beans was achieved by caecal expansion with highly significant increases in the masses of both tissue and contents (Table 3). Possible mechanisms for the hypertrophy which often accompanies complex carbohydrate consumption by rats (Wyatt *et al.* 1988; Rémésy & Demigné, 1989; Seal & Mathers, 1989) have been discussed by Goodlad & Mathers (1990) and Mathers & Dawson (1991) but this remains an area of uncertainty. The higher water contents of caecal and especially colonic digesta observed in the present study when feeding beans are probably due to a greater content of bacteria (Stephen & Cummings, 1980; Goodlad & Mathers, 1990) with their associated intracellular and extracellular water.

Supply of fermentation endproducts to the tissues

The major fermentation endproducts are the VFA which are rapidly and extensively absorbed (McNeil *et al.* 1978; Ruppin *et al.* 1980). Our calculations of the amounts of VFA absorbed (based on OM disappearance from the LB and caecal VFA proportions) indicated that total VFA absorption increased 5·3 times so that with the diet with the highest level of beans (BH4), 29 mol VFA/d were absorbed which is equivalent to 35 kJ metabolizable energy.

This additional VFA supply was reflected in increases in portal blood VFA concentration (highly significant for propionate and butyrate) and in the concentration of acetate in heart blood. In addition propionate was detected in heart blood from rats fed on beans and rose linearly ($P < 0·01$) as intake of beans increased. This is in contrast to the study of Goodlad and Mathers (1990) where, even with the peas fed at the highest rate, propionate could not be detected in rat heart blood. In the present study, the concentrations of this metabolite were low and close to the limit of detection by the method used so that the apparent between-studies difference may be due more to variation in instrument sensitivity than to biological effects. It would appear that the efficiency of the liver in removing portal-supplied VFA was in order butyrate > propionate > acetate.

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