British Journal of Nutrition (1990), **64**, 569–587

Large bowel fermentation in rats given diets containing raw peas (*Pisum sativum*)

BY J. S. GOODLAD AND J. C. MATHERS*

Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU

(*Received 9 February 1990 – Accepted 24 May 1990*)

The digestion of non-starch polysaccharides (NSP) was studied in rats given semi-purified diets containing 0–500 g raw peas (*Pisum sativum*)/kg. NSP were equally well digested at all inclusion levels with digestibilities for individual constituents ranging from 0·58 for xylans to 0·99 for arabinose-containing polymers with a total NSP digestibility of 0·79. Increasing the dietary pea inclusion rate increased the amount of substrate flowing to the large bowel (LB) and this was associated with marked increases in caecal tissue and contents masses, a reduction in caecal transit time from 0·88 to 0·43 d and a threefold increase in faecal bacterial biomass output. Caecal pH fell as did the molar proportions of acetate, isobutyrate, isovalerate and valerate whilst butyrate increased when peas were included in the diet. Possible mechanisms for these fermentation end-product changes are discussed. Pea inclusion in the diet was associated with increased volatile fatty acid and 3-hydroxy butyrate concentrations in portal and heart blood. It was concluded that peas are a rich source of fermentable polysaccharides which produce a LB fermentation pattern of potential health benefit.

Large bowel fermentation: Carbohydrate digestion: Peas: Rat

Fermentation of a wide range of exogenous and endogenous materials occurs in the large bowel (LB) of many animals, including man, with the production of volatile fatty acids (VFA), gases (carbon dioxide, hydrogen and methane) and bacterial biomass as major end-products. The VFA are readily absorbed (McNeil *et al.* 1978; Ruppin *et al.* 1980) and may be utilized for energy but are also thought to have specific effects on metabolism within the gut mucosa (Roediger, 1980; Ardawi & Newsholme, 1985; Sakata, 1987) and liver (Chen *et al.* 1984). Studies with ileostomists have shown that the amount and composition of material flowing into the LB and, therefore, potentially available as substrates for fermentation is readily altered by dietary means with starches (Chapman *et al.* 1985; Englyst & Cummings, 1985; Wolever *et al.* 1986), non-starch polysaccharides (NSP) (Englyst & Cummings, 1985) and proteins (Gibson *et al.* 1976; Chacko & Cummings, 1988) probably the major exogenous sources of LB substrate (Cummings & Englyst, 1987). Several studies in vivo with rats have shown that variation in the amount or types of NSP-containing foods results in an altered VFA pattern (Cheng *et al.* 1987; Key & Mathers, 1987), and limited information from incubations in vitro with faecal or LB contents suspensions (Englyst *et al.* 1987; Goodlad & Mathers, 1988) suggests that substrate supply may influence end-product formation. However, there is also evidence of substantial inter-individual variation in VFA production from a given substrate (Weaver *et al.* 1989) and it cannot be concluded that substrate supply is the only, or indeed the major, factor influencing LB fermentation pattern.

The present study was designed to test the capacity of the rat LB to ferment graded amounts of substrate supplied as raw peas (*Pisum sativum*) which are rich in slowly digested

* For reprints.

starch and NSP and, therefore, likely to be a good source of material which will escape digestion in the small intestine. Increasing the inclusion rate of peas in the diet had marked effects on fermentation pattern within the caecum and on concentrations of VFA in portal and heart blood. Pea complex carbohydrates appeared equally well digested at all levels of dietary inclusion.

A brief account of part of the present work has been published (Goodlad & Mathers, 1987).

MATERIALS AND METHODS

Animals and housing

Thirty-six male Wistar rats were purchased (A. Tuck & Sons, Essex), housed in stock cages (four rats/cage) and given *ad lib.* access to a stock diet (41B; Oxoid Ltd, Basingstoke, Hampshire) for a pre-experimental period of 6 d. Thereafter the rats (initial weight 217 (SE 1·3) g) were randomly allocated to individual Perspex and stainless-steel metabolism cages (Thompson, 1970) which permitted complete separation and collection of urine and faeces.

Diets and feeding

Six semi-purified diets were formulated (Table 1) to contain graded concentrations (0–500 g/kg) of milled raw peas (var. Progreta), with balancing alterations in sucrose and casein to maintain similar crude protein (nitrogen × 6·25) and calculated metabolizable energy (ME) contents. Autoclaved maize starch (the only source of complex carbohydrate in addition to peas) was present in equal concentrations in all diets as was the non-absorbed marker chromic oxide.

Each animal was offered at 10·00 hours daily, 18 g air-dry diet/d for the first 17 d and 20 g/d during the final 7 d of the experiment. Water was available *ad lib.* The diet allocation was usually consumed by 13·00 hours. Food residues were removed daily, dried and weighed.

Experimental protocol

Animals were weighed at the beginning and end of each balance period and on the day of slaughter. The first 10 d of the feeding period was for adaptation to the diets. Two consecutive 7 d balance periods followed during which food intake was measured and total collections of urine (into flasks containing 5 ml sulphuric acid (50 ml/l)) and faeces were made separately for each of the two 7 d periods. Faeces for each 7 d period were bulked separately, stored at −20° before being freeze-dried, weighed and milled for analyses. Pooled (7 d) urine samples were diluted with water to 250 ml and a portion stored at 4° awaiting analysis.

At the end of the second balance period, gastrointestinal and blood samples were obtained from all animals over a period of 3 d with twelve animals (two/diet) being sampled on each day. Sampling commenced at 14·00 hours daily and was completed in approximately 2·5 h. Each animal was anaesthetized with diethyl ether, a mid-line laparotomy was performed and blood samples collected from the portal vein (1·5 ml) and heart (5 ml) into heparinized syringes. Immediately, 100 μl portions of blood were mixed with 1 ml perchloric acid (30 ml/l) in preparation for 3-hydroxybutyrate (3OHB) analysis. The remaining blood was transferred to a stoppered glass tube, held on ice until the end of the sampling period and then stored at −20° to await VFA analysis. The caecum was removed, weighed full and the pH of its contents measured by inserting a microelectrode attached to a pH meter. Duplicate portions (approximately 0·5 g) of caecal digesta were mixed (1:0·5, w/v) with deproteinizing solution in preparation for VFA determination (Mathers *et al.* 1990) and the remaining digesta transferred to a pre-weighed tube, weighed, freeze-dried, re-weighed and ground for analysis. Caecal tissue was rinsed free of digesta,

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

Diet...	1	2	3	4	5	6
Raw peas (*Pisum sativum*)*	0	100	200	300	400	500
Maize starch†	398	398	398	398	398	398
Sucrose	386·5	307	228·9	151·9	74·3	0·6
Casein	150	129	106·6	83	60	33
Maize oil	33	33	33	33	33	33
L-Methionine	1	1·5	2	2·6	3·2	3·9
Vitamin and mineral mix‡	31·5	31·5	31·5	31·5	31·5	31·5
Analysed composition (g/kg DM)						
Crude protein (nitrogen × 6·25)	132	137	139	143	144	142
Total NSP	1·6	17·2	35·2	56·4	80·2	102·8
Cellulose	0·1	6	14	25	38	52
NCP	1·5	11·2	21·2	30·6	38·2	49·8
Arabinose	0	2·1	4·7	8·4	11·8	15·8
Xylose	0	0·9	2·7	3·2	5·2	6·2
Mannose	0	0·1	0·2	0·4	0·4	0·6
Galactose	0·3	0·7	2·6	2·6	3·3	4·7
Glucose	1·1	11·2	20·7	39·4	52·5	68·2
Uronic acids	0·1	2·1	3·1	5·8	7·1	7·4
Resistant starch	1·3	2·8	3·4	11·8	12·0	14·1
Total oligosaccharides	0	3·7	7·3	14·1	15·5	24·8
Raffinose	0	0·6	0·9	1·8	2·2	4·2
Stachyose	0	1·5	2·9	4·4	5·1	7·9
Verbascose	0	1·6	3·5	7·9	8·2	12·7
Calculated energy content MJ ME/kg	14·5	14·4	14·3	14·2	14·1	14·0

NSP, non-starch polysaccharide; NCP, non-cellulosic polysaccharide; ME, metabolizable energy.

* var. Progreta milled to pass a 1 mm screen.

† Autoclaved at 120° for 20 min, freeze-dried and milled to pass a 1 mm screen.

‡ Contained (g/kg premix): CaH_2PO_4 528, KCl 204, $MgSO_4$ 60, NaCl 38, $MnSO_4.4H_2O$ 6·6, $FeSO_4.7H_2O$ 5·1, $ZnCl_2$ 0·75, $CuCl_2$ 0·6, KIO_3 0·3 and (mg/kg premix): NaF 67, $CrCl_3$ 68, Rovimix AD_3 (Roche) 300, Rovimix E50 (Roche) 900, menadione 15, choline chloride 40, folic acid 30, niacin 600, calcium pantothenate 94, riboflavin 90, thiamin 120, pyridoxine hydrochloride 215, cyanocobalamin 1·5 plus 63·5 g Cr_2O_3 and sucrose to make 1 kg.

lightly blotted dry and weighed. The contents of the terminal one-sixth by length of the small intestine were flushed using 5 ml saline (9 g sodium chloride/l) into a sample pot, frozen at −20° and freeze-dried for analysis. A freshly voided faecal pellet, or a pellet from the terminal colon was collected for determination of faecal moisture content.

Analytical methods

NSP and its constituents were determined as described by Englyst & Cummings (1984) and resistant starch (RS) determined by omitting the dimethyl sulphoxide (DMSO) addition step. For determination of oligosaccharides, 5 g portions of diet or faeces were extracted with 200 ml aqueous ethanol (400 ml/l), clarified by addition of Carrez solutions (1 ml Carrez A (containing 219 g $Zn(CH_3COO)_2.2H_2O$ and 30 g glacial acetic acid/l) followed by 1 ml Carrez B (containing 106 g $K_4Fe(CN)_6.3H_2O$/l)) and a portion concentrated by rotary evaporation at 60°. The residue was taken up in a suitable volume of aqueous acetonitrile solution (300 ml/l) and individual oligosaccharides separated and quantified by high-performance liquid chromatography (HPLC) using a 150 mm × 4·9 mm i.d. column packed with Magnusil 8H bonded with aminopropyltriethoxy-silane (70 g/kg), elution with aqueous acetonitrile solution (730 ml/l) and detection by refractive index (Waters

R401). For determination of oligosaccharides in urine, samples (100 ml) were concentrated by freeze-drying, the residue dissolved in 1 ml acetonitrile solution (700 ml/l), passed through a 0·45 μm MF-Millipore filter and 25-μl portions of filtrate injected on to the HPLC column. N in diets, faeces and urine was measured by a Kjeldahl procedure, Cr_2O_3 and VFA in caecal digesta as described by Mathers *et al.* (1990) and 3OHB in deproteinized blood enzymically (Lloyd *et al.* 1978). For determination of blood VFA, portions (1 ml) of thawed whole blood were mixed with 5 ml propan-2-ol solution containing 100 μM-3-methyl valerate as internal standard. The precipitate was removed by centrifugation at 2500 ***g*** and 4°, and the supernatant fraction mixed with 200 μl 0·2 M-sodium hydroxide to form the sodium salts of the VFA. The propan-2-ol was evaporated off under a stream of air at 30° until the VFA salts were completely dry. At 20 min before analysis the salts were dissolved in 60 μl orthophosphoric acid solution (65 ml/l) and 1 μl injected into a fused silica capillary column (OV 351-15 N; Jones Chromatography Ltd, Llanbradach, Mid Glamorgan) in a Shimadzu GC (Kyoto, Japan). α,ϵ-Diaminopimelic acid (DAPA) in faecal samples was determined by automated ion-exchange chromatography (Locarte MK 4) after digestion with 6 M-hydrochloric acid (McMeniman & Armstrong, 1979).

Statistical methods

Data were examined by one-way analysis of variance and orthogonal polynomials used to describe the response to pea inclusion in the diets. Results are presented as means for each diet (n 6) with their standard errors based on the between-animal within-diets variation. The significance of linear, quadratic and cubic components of the response to the dietary inclusion level of peas is indicated in the Tables. Digestibility of pea polysaccharides was determined by linear regression of mouth-to-anus disappearance against intake and the relationship between portal and heart blood acetate concentrations by polynomial regression.

RESULTS

Diet composition

Analysed crude protein (nitrogen × 6·25) tended to increase while calculated ME contents of the diets tended to decrease slightly with increasing level of pea inclusion but the most marked changes were, as expected, in the dietary concentrations of NSP, RS and oligosaccharides (Table 1). Only traces of NSP (largely as pancreatin-resistant but 1 M-H_2SO_4-hydrolysable glucose-containing polymers) were detected in the diet without peas, with graded increases in dietary pea content reflected in increases in NSP and its constituent sugars (up to 102·8 g/kg diet), RS (up to 14·1 g/kg diet) and oligosaccharides (up to 24·8 g/kg diet). Dietary NSP were approximately equally divided between cellulose and non-cellulosic polysaccharides (NCP) with glucose > arabinose > uronic acids > xylose > galactose in the latter. Only small concentrations of mannose and no rhamnose were detected in the diets, although traces of rhamnose were detected in the peas used to formulate the diets (values not shown). Verbascose contributed approximately three times as much by weight, as did raffinose, to the oligosaccharide fraction, with stachyose intermediate between these two.

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

Food refusals were negligible for all rats during both balance periods and rat growth rates were good. In week 1, there were no between-diet differences in growth rate and, although rats given the five pea-containing diets gained more weight than those on the diet without peas in week 2, between-diet differences in weight gain were not significant when averaged over both weeks (Table 2). Food conversion ratio (FCR; g food DM eaten/g live weight

Table 2. *Growth rate (g/d) and food conversion ratio (FCR; g feed/g gain) of rats given semi-purified diets† containing graded levels of raw peas* (Pisum sativum *var. Progreta*)

(Means for six rats per diet)

Pea content of diet (g/kg)...	0	100	200	300	400	500	SEM	Statistical significance of dietary effects		
								Lin	Quad	Cub
Growth rate:										
Week 1	6·0	6·5	5·6	6·3	5·8	5·6	0·20	NS	NS	NS
Week 2	7·9	10·2	9·8	9·9	10·0	9·6	0·41	*	**	NS
Weeks 1+2	7·0	8·3	7·7	8·1	7·9	7·6	0·33	NS	NS	NS
FCR:										
Week 1	2·8	2·6	3·1	2·7	2·9	3·0	0·16	NS	NS	NS
Week 2	2·3	1·8	1·9	1·9	1·8	1·9	0·09	**	**	NS
Weeks 1+2	2·5	2·1	2·3	2·2	2·2	2·3	0·11	NS	*	NS

Lin, Quad and Cub are linear, quadratic and cubic effects of pea content of diet; NS, not significant.
* $P < 0·05$, ** $P < 0·01$.
† For details, see Table 1.

gain) was lower for pea-containing diets in week 2 and when weeks 1 and 2 were combined. The slightly higher concentration of N in the pea-containing diets and the higher food intakes in week 2 were reflected in higher N intakes but these were matched by marked linear ($P < 0·001$) increases in faecal N output in both weeks. Urinary N excretion appeared to be unaffected by diet and N retention was similar for all diets in both balance periods (Table 3).

Partition of dry matter (DM) digestion within the gut

Mouth-to-anus apparent digestibility was calculated from knowledge of DM intakes and faecal DM outputs (method 1) and also by the marker ratio technique (method 2). Both methods resulted in similar estimates of apparent digestibility of DM for both weeks (Table 4). Apparent DM digestibility for the diet without peas was high (0·97) and there were strong linear decreases in digestibility with increasing dietary inclusion of peas, but even with 500 g peas/kg diet DM apparent digestibility did not fall below 0·91. Using the marker ratio technique, apparent digestibility of DM was calculated for digesta samples taken from the terminal ileum and caecum. Ileal digestibility was lower than that determined on faeces, especially for the diets containing the higher levels of peas, but caecal and faecal values were similar. Faecal recovery of ingested Cr_2O_3 was complete (mean proportion recovered 1·06 (SE 0·029)) and flow of DM through the terminal ileum was calculated from knowledge of Cr_2O_3 intake and Cr_2O_3:DM ratio in ileal digesta. With the diet without peas, 1·2 g DM emptied from the ileum into the LB daily and this increased linearly with increasing dietary inclusion of peas to 2·7 g/d (Table 5). The difference between DM flow from the ileum and output in the faeces is the amount of DM which was apparently fermented in the LB, and this increased linearly and approximately doubled as the proportion of peas in the diet increased from 0 to 500 g peas/kg.

Caecal mass, pH and fermentation pattern

Caecal mass fell from 3·6 to 2·9 g with the first level of pea inclusion in the diet and then increased to reach a maximum of 5·4 g (Table 6) and a similar pattern was observed for caecal contents wet weight. There was a strong linear increase in caecal tissue mass, with

Table 3. *Nitrogen intake and output in urine and faeces, and retention (g/week) for rats given semi-purified diets† containing graded levels of raw peas* (Pisum sativum *var. Progreta*)

(Means for six rat per diet)

Pea content of diet (g/kg)...	0	100	200	300	400	500	SEM	Statistical significance of dietary effects: Lin	Quad	Cub
N intake:										
Week 1	2·49	2·59	2·62	2·67	2·68	2·62	0·003	***	***	***
Week 2	2·73	2·87	2·91	2·97	2·98	2·91	0·017	***	***	NS
Faecal N output:										
Week 1	0·16	0·19	0·29	0·33	0·38	0·40	0·017	***	NS	NS
Week 2	0·24	0·25	0·35	0·40	0·44	0·53	0·018	***	NS	NS
Urinary N output:										
Week 1	0·77	0·64	0·80	0·62	0·83	0·74	0·065	NS	NS	NS
Week 2	1·05	0·88	1·01	0·84	0·94	0·94	0·118	NS	NS	NS
N retention:										
Week 1	1·57	1·75	1·53	1·72	1·48	1·48	0·067	NS	NS	NS
Week 2	1·45	1·74	1·55	1·73	1·60	1·45	0·118	NS	NS	NS

Lin, Quad and Cub are linear, quadratic and cubic effects of pea content of diet; NS, not significant.
*** $P < 0·001$.
† For details, see Table 1.

Table 4. *Apparent dry matter (DM) digestibility measured at various sampling sites in rats given semi-purified diets† containing graded levels of raw peas* (Pisum sativum *var. Progreta*)

(Means for six rats per diet)

Pea content of diet (g/kg)...		0	100	200	300	400	500	SEM	Statistical significance of dietary effects: Lin	Quad	Cub
Digestibility; method 1											
Week 1		0·97	0·96	0·94	0·94	0·93	0·91	0·002	***	NS	NS
Week 2		0·97	0·96	0·94	0·92	0·92	0·91	0·004	***	**	NS
Sampling sites; method 2											
Terminal ileum	Week 2	0·94	0·91	0·90	0·89	0·85	0·85	0·009	***	NS	NS
Caecum	Week 2	0·96	0·95	0·93	0·93	0·91	0·91	0·003	***	NS	NS
Faeces:	Week 1	0·97	0·97	0·95	0·94	0·93	0·93	0·002	***	*	NS
	Week 2	0·97	0·96	0·94	0·92	0·92	0·91	0·003	***	NS	*

Method 1, digestibility calculated from total collection of faeces; method 2, digestibility calculated using the marker ratio technique; Lin, Quad and Cub are linear, quadratic and cubic effects of pea content of diet; NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details, see Table 1.

rats on the highest level of peas having 43% more caecal tissue than those given the diet without peas. The proportion of DM in caecal contents tended to fall when peas were included in the diet but faecal pellets from pea-fed rats had much higher DM contents than those given the diet without peas. Considerable desiccation of digesta occurred between the

Table 5. *Intestinal dry matter (DM) flows, DM apparent disappearance in, and estimated absorption of volatile fatty acids (VFA) from the large bowel of rats given semi-purified diets*† *containing graded levels of raw peas* (Pisum sativum *var. Progreta*)

(Means for six rats per diet)

Pea content of diet (g/kg)...	0	100	200	300	400	500	SEM	Statistical significance of dietary effects		
								Lin	Quad	Cub
DM flow from terminal ileum (g/d)	1·2	1·6	1·8	2·1	2·7	2·7	0·18	***	NS	NS
Faecal DM output (g/d; week 2)	0·6	0·8	1·0	1·2	1·3	1·7	0·03	***	**	NS
DM apparent disappearance in large bowel (g/d)	0·5	0·8	0·9	0·9	1·4	0·9	0·18	*	NS	NS
Calculated absorption of VFA (mmol/d)‡										
Acetate	2·2	3·4	3·2	3·4	4·9	3·4	0·63	*	NS	NS
Propionate	0·7	1·0	1·0	1·1	1·8	1·1	0·22	*	NS	NS
Butyrate	0·3	0·6	0·7	0·7	1·3	0·8	0·20	**	NS	NS
Total	3·2	5·0	4·9	5·3	7·9	5·4	1·03	*	NS	NS

Lin, Quad and Cub are linear, quadratic and cubic effects of pea content of diet; NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details, see Table 1.
‡ Calculated by conventional stoichiometry (Demeyer & Van Nevel, 1975) as described on p. 581 assuming that organic matter disappearance in the large bowel was 0·54 times measured DM disappearance (Goodlad, 1989) and using measured VFA proportions (Table 7).

caecum and distal colon. Caecal transit time (TT) was calculated as the amount of Cr_2O_3 recovered in that organ divided by Cr_2O_3 intake (Faichney, 1975) and was reduced by half with the lowest level of peas in the diet. Further increases in pea intake had no effect of caecal TT.

Caecal digesta pH fell steadily from 6·3 on the diet without peas to 5·9 in rats given 500 g peas/kg diet. Total VFA (mmol/kg caecal contents) increased sharply with the lowest level of inclusion of peas, with further increases in dietary pea content having much smaller effects (Table 7). Caecal VFA pattern (expressed as molar proportions of VFA) was strongly manipulated by diet with large linear increases in butyrate matched by falls in acetate, isobutyrate, isovalerate and valerate.

As an index of LB bacterial biomass production, faecal DAPA was measured. The concentration of DAPA in faecal DM was not significantly affected by diet but the total output increased 3-fold with increasing levels of inclusion of peas in the diet (Table 6). This additional bacterial N together with undigested pea protein may have contributed to the extra faecal N output reported in Table 3.

Digestibility of NSP, oligosaccharides and RS

Oligosaccharides were not detectable in faeces or urine from animals given the 500 g peas/kg diet; other dietary groups were not examined. Faecal concentrations of NSP and its components were generally higher than dietary concentrations and there were considerable differences in the pattern of NSP constituents between diet and faeces, most notably the much higher ratio of cellulose : NCP in the latter (Table 8). Both rhamnose and

Table 6. *Caecal mass, caecal tissue and contents weights, proportion of dry matter (DM) in caecal digesta and freshly voided faeces and α,ε-diaminopimelic acid (DAPA) in faeces from rats given semi-purified diets† containing graded levels of raw peas* (Pisum sativum *var. Progreta*)

(Means for six rats per diet except where indicated)

Pea content of diet (g/kg)...	0	100	200	300	400	500	SEM	Statistical significance of dietary effects		
								Lin	Quad	Cub
Caecal mass (g)	3·6	2·9	3·8	4·0	4·1	5·4	0·27	***	**	NS
Caecal tissue mass (g)	0·83	0·87	0·99	1·01	0·97	1·19	0·045	***	NS	NS
Caecal contents wet weight (g)	2·8	2·0	2·8	3·0	3·1	4·2	0·24	***	**	NS
Proportion of DM in caecal digesta	0·23	0·19	0·20	0·19	0·20	0·19	0·08	**	*	*
Proportion of DM in faecal pellet‡	0·31	0·45	0·44	0·47	0·42	0·42	0·009	*	***	*
Caecal transit time (d)	0·88	0·43	0·46	0·43	0·38	0·45	0·047	***	***	*
Faecal DAPA concentration (mg/g DM)	3·4	4·8	4·4	5·2	4·7	4·1	0·80	NS	NS	NS
Faecal DAPA output (mg/7 d)	16§	25	31	45 ‖	44	50	9·6 ‖	***	NS	NS

Lin, Quad and Cub are linear, quadratic and cubic effects of pea content of diet; NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details, see Table 1.
‡ Pellet taken from distal colon or freshly voided faecal pellet.
§ n 5; ‖ n 4.

mannose were present in measurable amounts in faeces, and for all NSP components (except mannose) faecal concentration increased with increasing levels of peas in the diet. Concentrations of RS in faeces when detected were small and not significantly different from zero. For pea-containing diets the NCP fraction contributed a higher proportion of the total digestible NSP than did cellulose. Cellulose was the major contributor to the NSP-glucose which was apparently digested, and for the other NSP-sugars and derivatives the amounts digested declined in the order arabinose > uronic acids > galactose > xylose > mannose. The apparent digestibilities of these dietary fractions in peas were calculated as the slopes of linear regressions of mouth-to-anus disappearance *v.* intake using individual values for all thirty-six rats (Table 9). All regressions were highly significant and the proportion of the variation accounted for by the regression was high for all components, falling below 0·9 for xylose and mannose only where the amounts apparently digested were comparatively small. The intercepts for all the measured carbohydrate fractions were close to zero. Almost 80% of the ingested NSP disappeared between the mouth and anus, with considerably higher apparent digestibilities for NCP than for cellulose. Of the NCP fraction arabinose was almost completely digested, and for the other components apparent digestibility declined in the order galactose > mannose > uronic acids > xylose. RS was completely digested.

Table 7. *pH, total volatile fatty acids (VFA) concentration (mmol/kg caecal contents) and molar proportions (mmol/mol) of individual VFA in the caeca of rats given semi-purified diets† containing graded levels of raw peas* (Pisum sativum *var. Progreta*)

(Means for six rats per diet)

Pea content of diet (g/kg)...	0	100	200	300	400	500	SEM	Statistical significance of dietary effects		
								Lin	Quad	Cub
Caecal pH	6·3	6·3	6·2	6·0	5·8	5·9	0·12	**	NS	NS
Total VFA (mmol/kg caecal content)	118	157	159	162	163	166	7·3	***	*	NS
Molar proportions of individual VFA										
Acetate	647	667	627	624	606	619	36·2	*	NS	NS
Propionate	207	193	198	207	216	204	9·1	NS	NS	NS
Isobutyrate	12	11	11	10	8	8	1·7	**	NS	NS
Butyrate	99	102	139	135	147	149	22·7	**	NS	NS
Isovalerate	15	14	12	11	11	9	1·1	*	NS	NS
Valerate	19	13	13	12	11	10	2·1	*	NS	NS

Lin, Quad and Cub are linear, quadratic and cubic effects of pea content of diet; NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details, see Table 1.

Table 8. *Concentrations (g/kg dry matter (DM)) of non-starch polysaccharide (NSP), non-cellulosic polysaccharide (NCP), cellulose, uronic acids and the constituent sugars of the NSP fraction and resistant starch in the faeces of rats given semi-purified diets† containing graded levels of raw peas* (Pisum sativum *var. Progreta*)

(Means for six rats per diet)

Pea content of diet (g/kg)...	0	100	200	300	400	500	SEM	Statistical significance of dietary effects		
								Lin	Quad	Cub
Total NSP	17	120	149	217	249	259	12·2	***	***	NS
NCP	14	46	54	75	77	77	4·9	***	***	NS
Cellulose	2	74	96	142	172	182	9·2	***	**	NS
Constituent sugars of NSP fraction										
Rhamnose	1	2	1	3	3	3	0·7	*	NS	NS
Arabinose	Tr	1	1	3	2	2	0·6	*	NS	NS
Xylose	2	15	18	26	31	29	2·6	***	**	NS
Mannose	2	1	2	1	2	1	0·4	NS	NS	NS
Galactose	3	7	6	8	8	7	0·8	**	**	NS
Glucose	9	88	108	155	185	195	9·2	***	***	NS
Uronic acids	Tr	10	13	19	17	20	1·5	***	**	NS
Resistant starch	0	4	−1	0	−3	−5	4·4	NS	NS	NS

Lin, Quad and Cub are linear, quadratic and cubic effects of pea content of diet; Tr, trace (< 0·5 g/kg); NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details, see Table 1.

Table 9. *Apparent digestibilities* (B) *of non-starch polysaccharides* (*NSP*) *fraction and its constituents, and of resistant starch* (*RS*), *calculated from linear regressions of the form* $y = A + Bx$ *for mouth-to-anus disappearance* (y) v. *intake* (x) *for rats given semi-purified diets containing graded levels of raw peas* (Pisum sativum *var. Progreta*) *as the only dietary source of NSP*

(Each regression was calculated using individual values for all thirty-six rats)

	A	SE	*B*	SE	R^2
NSP	−0·004	0·0075	0·789	0·0010	0·995
NCP	−0·004	0·0031	0·901	0·0008	0·997
Cellulose	−0·008	0·0051	0·682	0·0014	0·987
Arabinose	0·000	0·0003	0·989	0·0003	1·000
Xylose	0·002	0·0018	0·583	0·0038	0·873
Mannose	0·000	0·0002	0·840	0·0051	0·889
Galactose	0·002	0·0006	0·888	0·0017	0·988
Glucose	−0·003	0·0052	0·741	0·0010	0·994
Uronic acids	0·000	0·0119	0·781	0·0018	0·982
RS	−0·002	0·0033	1·029	0·0028	0·975

NCP, non-cellulosic polysaccharides.

Table 10. *Concentrations of volatile fatty acids* (*VFA*; μM) *and of 3-hydroxybutyrate* (*3OHB*; μM) *in whole blood from six rats given semi-purified diets*† *containing graded levels of raw peas* (Pisum sativum *var. Progreta*)

(Means for six rats per diet)

Pea content of diet (g/kg)...	0	100	200	300	400	500	SEM	Statistical significance of dietary effects		
								Lin	Quad	Cub
Portal blood										
Acetate	475	500	507	697	842	868	51·6	***	NS	NS
Propionate	62	88	129	178	206	199	23·3	***	NS	NS
Butyrate	12	28	75	133	132	161	28·8	***	NS	NS
3OHB	22	42	44	56	61	67	8·2	***	NS	NS
Heart blood										
Acetate	272	286	272	331	425	462	39·5	***	NS	NS
3OHB	57	75	79	77	83	90	6·6	**	NS	NS

Lin, Quad and Cub are linear, quadratic and cubic effects of pea content of diet; NS, not significant.
** $P < 0·01$, *** $P < 0·001$.
† For details, see Table 1.

VFA and 3OHB in portal and heart blood

All three major VFA (acetate, propionate and butyrate) present in caecal contents were detected in portal blood, and there were very highly significant ($P < 0·001$) increases in the concentrations of each with increasing levels of inclusion of peas in the diet (Table 10). Acetate, the only VFA detected in blood withdrawn from the heart, was present at about half the concentrations found in portal blood and increased as pea intake increased. 3OHB concentrations also rose with increasing levels of peas in the diet, with heart blood having higher concentrations than portal blood.

DISCUSSION

The raw peas used in the present study contained relatively small concentrations of anti-nutritional factors (lectins and α-amylase inhibitor; Goodlad, 1989), were well accepted by the rats and resulted in equally good growth rates and N retention for pea-containing and basal diets.

Caecal hypertrophy

The mass of an intestinal organ may increase due to increases in the weights of tissue or digesta contents, or both. Increases in the tissue may result from greater weights of the mucosa or of the underlying muscle layers but most nutritional studies have concentrated on the mucosa. Mucosal hypertrophy will occur if the rate of epithelial proliferation relative to that of cell loss is increased. Diets rich in complex carbohydrates (NSP or starch resistant to α-amylase (*EC* 3.2.1.1)) often provoke hypertrophy of the LB in rats (Wyatt *et al.* 1988; Rémésy & Demigné, 1989; Seal & Mathers, 1989) and the present study was no exception. The greater mass of caecal and, sometimes, colonic tissue is associated with an increased mass of LB contents, but the mechanism(s) for this trophic effect has yet to be established. In the present study we did not distinguish between growth of the mucosa and of the underlying muscle. Increased mucosal growth with the pea-containing diets may have been stimulated by the extra VFA (Table 5) produced with these diets (Sakata, 1987), but in another study we found that 5-fold increases in VFA absorption produced by including haricot beans (*Phaseolus vulgaris*) in a wholemeal-bread-based diet had no effect on gut epithelial proliferation rate in rats (Key & Mathers, 1989). Since substantial caecal enlargement occurs in germ-free rats, even on an elemental diet (Goodlad *et al.* 1989), and in conventional animals given carboxymethylcellulose (which is essentially undegraded during passage through the gut) (Wyatt *et al.* 1988), clearly, VFA are not the only stimuli causing LB hypertrophy, and Wyatt *et al.* (1988) have argued 'that the caecum enlarges to accommodate the tendency of residual material to accumulate within it, and this is governed by the pattern of motility and bulk flow through the whole large bowel'. Further research is needed to explain why in some circumstances food and endogenous residues accumulate in the LB and to what extent this is responsible for, or a consequence of, tissue hypertrophy.

Digestion of NSP

The intestinal site(s) at which dietary NSP were digested was not investigated in the present study. In man it appears that all, or virtually all, the NSP which disappears within the gut does so within the LB as a result of fermentation (Cummings & Englyst, 1987) and the same may apply to the rat. However, in the pig it is clear that disappearance of NSP components can occur before the terminal ileum (Keys & DeBarthe, 1974; Sambrooke, 1979; Millard & Chesson, 1984; Goodlad, 1989).

The basal (without peas) diet appeared to contain small but detectable amounts of NSP (Table 1), mainly as glucose resistant to α-amylase but hydrolysable in 1 M-H_2SO_4 solution, despite being designed to provide no polysaccharides other than starch. These NSP may have been contaminants of the starch used for diet formulation. NSP were also detected in faeces from rats given the diet without peas (Table 8) at concentrations which were approximately 10-fold greater than those measured in the diet (Table 1). These faecal polymers may have been undigested dietary material, but are also likely to include endogenous or bacterially synthesized sugars (Stephen & Cummings, 1980; Longstaff & McNab, 1987).

Linear regression of mouth-to-anus disappearance *v.* intake for NSP and its constituents showed strong linear relationships with coefficients of determination better than 0·87, and

for most components better than 0·98. These findings indicate that the rat was able to digest pea NSP equally well at all levels of inclusion in the diet and, assuming that most (if not all) of this digestion occurred by fermentation in the LB, may have been assisted by the observed caecal hypertrophy (Table 6) which allowed increasing amounts of food residues to be accommodated within this organ. It is interesting to note, however, that with the diet containing 100 g peas/kg, caecal TT was decreased to half that on the basal diet but was not further altered when the proportion of peas in the diet was varied from 100 to 500 g/kg; caecal TT for all five pea-containing diets was approximately 10 h. A similar phenomenon was reported in man when graded levels of bran-enriched bread were eaten (Stephen *et al.* 1986). We conclude that the caecum responded to the increased substrate supply by increasing its size, and that this permitted equi-effective salvage of nutrients by fermentation without any increase in the time available for fermentation.

Almost 0·8 of the ingested pea NSP disappeared within the rat gut compared with estimates of 0·22 and 0·38 for NSP digestibility of the same variety of peas tube-fed to adult cockerels (Longstaff & McNab, 1987). Since rats appear to digest NSP to about the same extent as does man (Nyman *et al.* 1986), these findings indicate that peas may be a widely accepted, rich source of fermentable NSP for human consumption. Indeed, Williams & Olsted (1936) reported digestibilities of 0·45 and 0·80 for the cellulose and NCP in peas eaten by young men. In the present study, cellulose digestibility was 0·68 and that of NCP 0·90. Of the NCP constituent sugars, xylose was the least digestible (0·58) whilst arabinose-containing polymers were virtually completely digested (0·99). NCP-glucan was more digestible than cellulose. Dietary xylose:arabinose ratio was approximately 0·4:1 but for faeces was 18:1. Although absolute digestibilities were much lower, relatively high digestibility of pea arabinose compared with xylose was observed in the cockerels studied by Longstaff & McNab (1987). Graham *et al.* (1985) determined the digestibility of whole-crop-pea NSP in pigs by including samples in nylon bags inserted into the gut via a duodenal cannula and recovering the bag in faeces. Total NSP digestibility (0·49) was lower than for the pea seeds fed to rats in the present study or to pigs (Goodlad, 1989), but the relatively poor digestibility of xylans (0·23) when compared with arabinose-containing polymers (0·90) was clearly evident.

Much of the arabinose in peas is present as arabinose-containing pectic substances in the cell walls of cotyledons, whilst pea hulls are rich in cellulose and xylans (Selvendran, 1984; Longstaff & McNab, 1989). In chickens, pea hulls are very much less digestible (Longstaff & McNab, 1989) than the whole pea seeds (Longstaff & McNab, 1987), possibly because of the lignification of pea hulls (Reichert, 1981; Selvendran, 1984). The same is likely to be true for rats and would explain the relative indigestibility of cellulose and xylose in the present experiment.

Caecal fermentation pattern

Increased intake of peas was associated with a linear increase in the proportion of butyrate in caecal VFA matched by linear decreases in the proportions of acetate, isobutyrate, isovalerate and valerate with no change in the molar proportion of propionate. The source of this extra butyrate is of considerable interest. In vitro fermentation studies using human faeces (Macfarlane & Englyst, 1986) and pig LB contents (Goodlad & Mathers, 1988) have found high proportions of butyrate in fermentation end-products when starch is supplied as substrate. High proportions of butyrate were also observed in human stools when starch malabsorption within the small intestine was induced by ingestion of the α-glucosidase (*EC* 3.2.1.20) inhibitor acarbose, resulting in substantially increased LB fermentation of starch (Scheppach *et al.* 1988), and in caecal contents from rats fed on amylomaize, a source of starch relatively resistant to pancreatic amylase (Mallett *et al.* 1988). Compared with

cereal starch, starch from legumes such as peas is relatively slowly digested (Fleming & Vose, 1979; Snow & O'Dea, 1981; Longstaff & McNab, 1987) and contains a portion which escapes small intestinal digestion (Faulks *et al.* 1989). It is not clear whether the latter is equivalent to that starch fraction, here termed RS (cf. Englyst & Cummings, 1987), which is made accessible to α-amylase after treatment with DMSO (Table 1), but it is likely that increasing the proportion of peas in the diet resulted in more starch being made available for fermentation by LB bacteria. No RS was detected in faeces so that it is possible that this extra starch fermentation was responsible for some, at least, of the increased butyrate production with the pea-containing diets. However, high proportions of caecal butyrate have also been observed with other substrates, including guar gum (Tulung *et al.* 1987) and various wheat bran components (Cheng *et al.* 1987; Key & Mathers, 1987; Walter *et al.* 1988) and with *ad lib. v.* restricted feeding (Illman *et al.* 1986). In addition to starch, the peas supplied the LB with substantial amounts of NSP, nearly 80% of which was fermented, with oligosaccharides which were entirely fermented and, possibly, with protein. At present, there is no means of predicting the fermentation pattern which will result from such a mixture of substrates.

Substrate supply is not necessarily the only or indeed the major influence on fermentation pattern. Consumption of the pea-containing diets was associated with reductions in caecal pH and TT and both these factors are known to alter fermentation end-products without any change in substrate supplied (Finlayson, 1986; Silley & Armstrong, 1984). It is well-established from work with ruminants (Leng, 1970) and rabbits (Parker, 1976; Woodnutt, 1984) that during anaerobic fermentation within the gut there is substantial interconversion between acetate and butyrate, with a large proportion of butyrate derived from the acetate pool. In a highly competitive environment such as the LB it would seem at first sight disadvantageous to convert acetate to butyrate since this requires the expenditure of 1 mol ATP for activation of acetate/mol butyrate formed. However, in doing so the micro-organisms responsible are able to dispose of 2 mol of reducing equivalent and so permit glycolysis to proceed, resulting in a net gain of 1 mol ATP from substrate-level phosphorylation (Leng, 1970). Other routes for disposal of reducing equivalents (2H) include the production of hydrogen or methane gas, or both, and the reduction of ions such as sulphate and nitrate. In a three-stage continuous culture system with human faeces as inoculum (Gibson *et al.* 1988), methane production was considerably higher in the second and third vessels which were characterized by higher pH (6·5 and 7·0 respectively) and lower dilution rates (equivalent to TT of 0·87 and 1·23 d respectively). Such conditions were found in the present study only with the diet without peas and we suggest that the selective pressure produced with the pea-containing diets by the simultaneous increase in substrate supply and reductions in pH and TT may have encouraged the use of alternative routes for re-oxidation of reduced dinucleotides.

Changes in the proportions of branched-chain VFA and in valerate are probably the net result of a shift in the balance between the production of these acids by amino acid breakdown (El-Shazly, 1952; Rasmussen *et al.* 1988) and their utilization for *de novo* amino acid and, hence, bacterial protein synthesis (Russel & Hespell, 1981). The increased faecal DAPA output suggested a possible 3-fold increase in LB bacterial biomass production assuming that the DAPA concentration in bacterial cells was unaltered by diet.

Estimates of VFA absorption

Our estimates of VFA absorption from the LB (Table 5) are based on knowledge of organic matter (OM) disappearance between the terminal ileum and faeces and the pattern of VFA in caecal contents, assuming that the OM was largely carbohydrate and could be

Table 11. *Estimates of volatile fatty acid (VFA) absorption* (μmol/min) measured by arterio-venous difference and organic matter disappearance from the large bowel of rats given various diets*

Method used	Diet description	Estimated VFA absorption	Source
Arterio-venous difference			
	Stock diet	3·6†	Buckley & Williamson (1977)
	Starved 48 h	1·3†	
	Neomycin sulphate-treated	0†	
	Fibre-free	1·0‡	Demigné & Rémésy (1985)
	High-fibre	17·5‡	
	Fibre-free	2·8	Demigné *et al.* (1986*a*)
	High-fibre	24·5	
	Fibre-free	1·0‡	Rémésy & Demigné (1989)
	Lactulose (100 g/kg)	6·6‡	
	Pectin (100 g/kg)	6·6‡	
	Guar gum (100 g/kg)	7·0‡	
	Amylomaize starch:		
	250 g/kg	7·6‡	
	500 g/kg	16·3‡	
Organic matter disappearance			
	Fibre-free	2·2	Present study
	Peas (*Pisum sativum*) (g/kg):		
	100	3·5	
	200	3·4	
	300	3·7	
	400	5·5	
	500	3·8	

* Acetate + propionate + butyrate (except where indicated).
† Acetate only. Literature values for portal blood flow assumed by original authors.
‡ Caecum only.

represented as anhydrous hexose, and using conventional stoichiometric equations (Demeyer & Van Nevel, 1975). These estimates will be tenable provided: (1) relative rates of absorption of individual VFA are not markedly different from relative concentrations in the caecum, (2) the yields of VFA per unit mass of OM actually fermented are similar to those for a hexose polymer and (3) insignificant quantities of OM are added to digesta during passage through the LB. It should be noted that this calculation will underestimate VFA production to the extent that VFA are used as substrates for net microbial biomass production.

In Table 11, our estimates of VFA absorption are compared with values obtained by other workers using the difference in VFA concentration between portal vein and arterial blood multiplied by portal blood flow-rate. Despite the different methodology it is encouraging that the two procedures yield comparable results. It is also clear that dietary manipulations which alter the flow of substrate to the LB have marked effects on the supply of VFA to splanchnic tissues.

Fate of absorbed VFA

Tissue utilization of VFA is dependent on initial activation by fatty acyl-CoA synthetases which are present in many organs including the gut mucosa, liver, muscle, adipose tissue and brain (Groot *et al.* 1976). In ruminants, the rumen epithelial butyryl-CoA synthetase

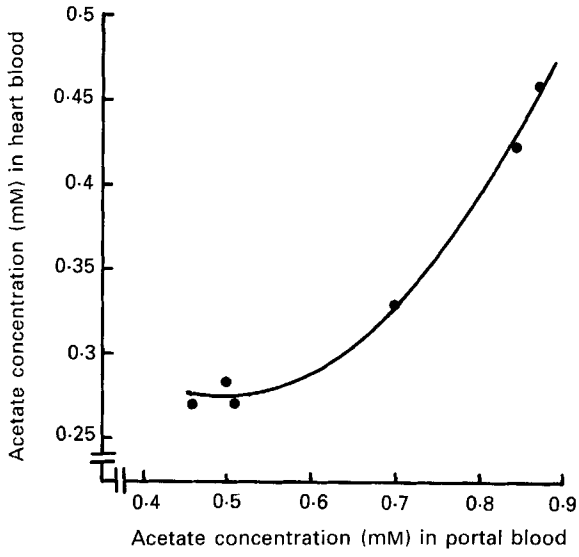

Fig. 1. Curvilinear relationship between the concentration of acetate in portal (x) and heart (y) blood from rats given semi-purified diets containing raw peas (*Pisum sativum*). Each value is the mean for six rats on each diet. The fitted line is: $y = 0{\cdot}561 - 1{\cdot}18x + 1{\cdot}22x^2$ $F(2, 3)$ 222, R^2 0·99.

(*EC* 6.2.1.2) is much more active than the CoA-synthetases (*EC* 6.2.1.1) for acetate and propionate (Ash & Baird, 1973) and a similar phenomenon has been reported for rat colonic tissue (Roediger, 1982). Butyrate is readily oxidized by isolated rat colonocytes (Roediger, 1982; Ardawi & Newsholme, 1985) but comparison of the molar ratios of the three major VFA in caecal contents and in portal blood in the present study did not indicate any consistent use of butyrate by the LB mucosa. If net uptake into the portal vein is calculated by subtracting heart blood concentrations (assumed to represent arterial supply) from portal concentrations, then the molar proportion of butyrate is lower than that in the caecal contents for diets containing 0 and 100 g peas/kg only. Further information on the extent of utilization of acetate and propionate by rat gut tissue is needed.

The activity of liver acetyl-CoA synthetase is reported to be approximately 0·5 μmol/min per g wet weight in rats (Aas, 1971) and cattle (Ash & Baird, 1973) with activities for propionyl- and butyryl-CoA synthetases between two and eight times higher in both species. Assuming a portal blood flow of 17 ml/min (Demigné *et al.* 1986*a*) for our rats, the liver would be capable of removing, in one pass, all the propionate and butyrate supplied via the portal vein on all diets, up to 0·8 of the portal acetate supply with the diet without peas but only 0·4 of the acetate reaching that organ in rats fed on the diet with the highest level of peas. This is confirmed by the lack of detectable propionate and butyrate in heart blood and by the curvilinear increase in heart acetate concentration with increases in portal acetate concentration (Fig. 1). Demigné *et al.* (1986*a*) also observed that the rat liver removed virtually all the afferent supply of propionate and butyrate but only 0·16 (fibre-free diet) to 0·24 (high-fibre diet) of acetate supply. Using rat livers perfused *in situ* with whole rat blood containing a range of acetate concentrations, Snoswell *et al.* (1982) found net hepatic production of acetate when the blood supply contained less than 0·25 μmol acetate/ml, whilst above this concentration there was net uptake with a fractional extraction of 0·4. There was no net acetate uptake by the liver in fed sheep studied by Bergman (1975), a finding confirmed by Demigné *et al.* (1986*b*) using sheep hepatocytes. The functional significance of the efficient removal by the liver of propionate and butyrate

but not acetate has not been clearly established, but may relate to prevention of narcosis which can be induced by injection of the longer-chain VFA (Samson *et al.* 1956).

Concentrations of 3OHB were higher in blood drawn from the heart than from the portal vein (Table 10), which suggests net utilization by the gut mucosa, confirming observations that this ketone may be a respiratory fuel for both small (Windmuller & Spaeth, 1980) and large intestinal mucosa (Roediger, 1982; Ardawi & Newsholme, 1985). The increase in circulating concentrations of 3OHB with increasing proportions of peas in the diet may indicate greater hepatic production of this ketone from the additional butyrate supplied via the portal vein from LB fermentation.

Conclusions

Peas are a relatively concentrated source of slowly digested starch and of fermentable NSP and oligosaccharides, all of which contribute to enhanced fermentation in the LB resulting in reduced lumen pH, reduced TT and increased absorption of acetate, propionate and especially butyrate. Each of these effects is potentially beneficial to the human consumer. Acidification of LB contents, reduced TT and increased butyrate production may help prevent the development of colo-rectal cancer (Cummings & Branch, 1982). Increased absorption of VFA, in place of sugars, may reduce hepatic lipogenesis (Pedroso *et al.* 1989, 1990), with apparently specific effects of propionate in reducing cholesterol production through an inhibitory action on the key regulatory enzyme for cholesterol synthesis, hydroxymethylglutaryl-CoA reductase (*EC* 1.1.1.88) (Rodwell *et al.* 1976; Chen *et al.* 1984).

J. S. G. was in receipt of a SERC CASE award in co-operation with Unilever Research, Colworth Laboratory, Bedford and the authors thank P. J. Evans and R. Mawson for their interest and provision of an HPLC column for oligosaccharide separation. Advice on portal blood sampling from D. S. Parker is also appreciated.

REFERENCES

Aas, M. (1971). Organ and subcellular distribution of fatty acid activating enzymes in the rat. *Biochimica et Biophysica Acta* **231**, 32–47.

Ardawi, M. S. M. & Newsholme, E. A. (1985). Fuel utilization in colonocytes of the rat. *Biochemical Journal* **231**, 713–719.

Ash, R. & Baird, G. D. (1973). Activation of volatile fatty acids in bovine liver and rumen epithelium. Evidence for control by autoregulation. *Biochemical Journal* **136**, 311–319.

Bergman, E. N. (1975). Production and utilization of metabolites by the alimentary tract as measured in portal and hepatic blood. In *Digestion and Metabolism in the Ruminant*, pp. 292–305 [I. W. McDonald and A. C. I. Warner, editors]. Armidale: University of New England Publishing Unit.

Buckley, B. M. & Williamson, D. H. (1977). Origins of blood acetate in the rat. *Biochemical Journal* **166**, 539–545.

Chacko, A. & Cummings, J. H. (1988). Nitrogen losses from the human small bowel: obligatory losses and the effect of physical form of food. *Gut* **29**, 809–815.

Chapman, R. W., Sillery, J. K., Graham, M. M. & Saunders, D. R. (1985). Absorption of starch by healthy ileostomates: effect of transit time and of carbohydrate load. *American Journal of Clinical Nutrition* **41**, 1244–1248.

Chen, W. L., Anderson, J. W. & Jennings, D. (1984). Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibres in cholesterol-fed rats. *Proceedings of the Society for Experimental Biology and Medicine* **177**, 372–376.

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.

Cummings, J. H. & Branch, W. J. (1982). Postulated mechanisms whereby fiber may protect against large bowel cancer. In *Dietary Fiber in Health and Disease*, pp. 313–325 [G. V. Vahouny and D. Kritchevsky, editors]. London: Plenum Press.

Cummings, J. H. & Englyst, H. N. (1987). Fermentation in the human large intestine and the available substrates. *American Journal of Clinical Nutrition* **45**, 1243–1245.

Demeyer, D. I. & Van Nevel, C. J. (1975). Methanogenesis, an integrated part of carbohydrate fermentation, and

its control. In *Digestion and Metabolism in the Ruminant*, pp. 366–382 [I. W. McDonald and A. C. I. Warner, editors]. Armidale: University of New England Publishing Unit.
Demigné, C. & Rémésy, C. (1985). Stimulation of absorption of volatile fatty acids and minerals in the cecum of rats adapted to a very high fiber diet. *Journal of Nutrition* **115**, 53–60.
Demigné, C., Yacoub, C. & Rémésy, C. (1986*a*). Effects of absorption of large amounts of volatile fatty acids on rat liver metabolism. *Journal of Nutrition* **116**, 77–86.
Demigné, C., Yacoub, C., Rémésy, C. & Fafournoux, P. (1986*b*). Propionate and butyrate metabolism in rat or sheep hepatocytes. *Biochimica et Biophysica Acta* **875**, 535–542.
El-Shazly, K. (1952). Degradation of protein in the rumen of sheep. II. Amino acid degradation by washed suspensions of rumen bacteria. *Biochemical Journal* **51**, 647–653.
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 Ltd.
Englyst, H. N., Hay, S. & Macfarlane, G. T. (1987). Polysaccharide breakdown by mixed populations of human faecal bacteria. *FEMS Microbiological Letters* **45**, 163–171.
Faichney, G. J. (1975). The use of markers to partition digestion within the gastro-intestinal tract of ruminants. In *Digestion and Metabolism in the Ruminant*, pp. 277–291 [I. W. McDonald and A. C. I. Warner, editors]. Armidale: University of New England Publishing Unit.
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.
Finlayson, H. J. (1986). The effect of pH on the growth and metabolism of *Streptococcus bovis* in continuous culture. *Journal of Applied Bacteriology* **61**, 201–208.
Fleming, S. E. & Vose, J. R. (1979). Digestibility of raw and cooked starches from legume seeds using the laboratory rat. *Journal of Nutrition* **109**, 2067–2075.
Gibson, G. R., Cummings, J. H. & Macfarlane, G. T. (1988). Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. *Applied and Environmental Microbiology* **54**, 2750–2755.
Gibson, J. A., Sladen, G. E. & Dawson, A. M. (1976). Protein absorption and ammonia production: the effects of dietary protein and removal of the colon. *British Journal of Nutrition* **35**, 61–65.
Goodlad, J. S. (1989). Digestion and large intestinal fermentation of pea (*Pisum sativum*) carbohydrates. PhD Thesis, University of Newcastle upon Tyne.
Goodlad, J. S. & Mathers, J. C. (1987). Digesta flow from the ileum and transit time through the caecum of rats given diets containing graded levels of peas. *Proceedings of the Nutrition Society* **46**, 149A.
Goodlad, J. S. & Mathers, J. C. (1988). Effects of food carbohydrates on large intestinal fermentation in vitro. *Proceedings of the Nutrition Society* **47**, 176A.
Goodlad, R. A., Ratcliffe, B., Fordham, J. P. & Wright, N. A. (1989). Does dietary fibre stimulate intestinal epithelial cell proliferation in germ free rats? *Gut* **30**, 820–825.
Graham, H., Åman, P., Newman, R. K. & Newman, C. W. (1985). Use of a nylon-bag technique for pig feed digestibility studies. *British Journal of Nutrition* **54**, 719–726.
Groot, P. H. E., Scholte, H. R. & Hülsmann, W. C. (1976). Fatty acid activation: specificity, localisation and function. *Advances in Lipid Research* **14**, 75–126.
Illman, R. J., Topping, D. L. & Trimble, R. P. (1986). Effects of food restriction and starvation–refeeding on volatile fatty acid concentrations in the rat. *Journal of Nutrition* **116**, 1694–1700.
Key, F. B. & Mathers, J. C. (1987). Response of rat caecal metabolism to varying proportions of white and wholemeal bread. *Proceedings of the Nutrition Society* **46**, 11A.
Key, F. B. & Mathers, J. C. (1989). Effects on volatile fatty acid production and gut epithelial proliferation of adding haricot beans to a wholemeal bread diet. *Proceedings of the Nutrition Society* **48**, 47A.
Keys, J. E. Jr & DeBarthe, J. V. (1974). Cellulose and hemicellulose digestibility in the stomach, small intestine and large intestine of swine. *Journal of Animal Science* **39**, 53–56.
Leng, R. A. (1970). Formation and production of volatile fatty acids in the rumen. In *Physiology of Digestion and Metabolism in the Ruminant*, pp. 406–421 [A. T. Philipson, editor]. Newcastle upon Tyne: Oriel Press.
Lloyd, B., Burrin, J., Smythe, P. & Alberti, K. G. M. M. (1978). Enzymic fluorometric continuous-flow assays for blood glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate. *Clinical Chemistry* **34**, 1724–1729.
Longstaff, M. & McNab, J. M. (1987). Digestion of starch and fibre carbohydrates in peas by adult cockerels. *British Poultry Science* **28**, 261–285.
Longstaff, M. & McNab, J. M. (1989). Digestion of fibre polysaccharides of pea (*Pisum sativum*) hulls, carrot and cabbage by adult cockerels. *British Journal of Nutrition* **62**, 563–577.
Macfarlane, G. T. & Englyst, H. N. (1986). Starch utilization by the human large intestinal microflora. *Journal of Applied Bacteriology* **60**, 195–201.

McMeniman, N. P. & Armstrong, D. G. (1979). The flow of amino acids into the small intestine of cattle when fed heated and unheated beans (*Vicia faba*). *Journal of Agricultural Science, Cambridge* **93**, 181–188.
McNeil, N. I., Cummings, J. H. & James, W. P. T. (1978). SCFA absorption by the human large intestine. *Gut* **19**, 819–822.
Mallett, A. K., Bearne, C. A., Young, P. J., Rowland, I. R. & Berry, C. (1988). Influence of starches of low digestibility on the rat caecal microflora. *British Journal of Nutrition* **60**, 597–604.
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.
Millard, P. & Chesson, A. (1984). Modification to swede (*Brassica napus* L.) anterior to the terminal ileum of pigs: some implications for the analysis of dietary fibre. *British Journal of Nutrition* **52**, 583–594.
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.
Parker, D. S. (1976). The measurement of production rates of volatile fatty acids in the caecum of the conscious rabbit. *British Journal of Nutrition* **36**, 61–70.
Pedroso, L. M. R., Finlayson, H. J. & Mathers, J. C. (1989). Effects of dietary wheat bran on activities of key enzymes of lipid and carbohydrate metabolism in rat liver. *Proceedings of the Nutrition Society* **48**, 54A.
Pedroso, L. M. R., Mathers, J. C. & Finlayson, H. J. (1990). Effects of feeding raw peas on activities of key enzymes of lipid and carbohydrate metabolism in rat liver. *Proceedings of the Nutrition Society* **49**, 52A.
Rasmussen, H. S., Holtug, K. & Mortensen, P. B. (1988). Degradation of amino acids to short-chain fatty acids in humans. An in vitro study. *Scandinavian Journal of Gastroenterology* **23**, 178–182.
Reichert, R. D. (1981). Quantitative isolation and estimation of cell wall material from dehulled pea (*Pisum sativum*) flours and concentrates. *Cereal Chemistry* **58**, 266–270.
Rémésy, C. & Demigné, C. (1989). Specific effects of fermentable carbohydrates on blood urea flux and ammonia absorption in the rat cecum. *Journal of Nutrition* **119**, 560–565.
Rodwell, V. W., Nordstrom, J. L. & Mitschelen, J. J. (1976). Regulation of HMG CoA reductase. *Advances in Lipid Research* **14**, 1–74.
Roediger, W. E. W. (1980). Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa of man. *Gut* **21**, 793–798.
Roediger, W. E. W. (1982). Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* **83**, 424–429.
Ruppin, H., Bar-Meir, S., Soergel, K. H. & Schmitt, M. G. (1980). Absorption of SCFA by the colon. *Gastroenterology* **78**, 1500–1507.
Russel, J. B. & Hespell, R. B. (1981). Microbial rumen fermentation. *Journal of Dairy Science* **64**, 1153–1160.
Sakata, T. (1987). Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. *British Journal of Nutrition* **58**, 95–103.
Sambrooke, I. E. (1979). Studies on digestion and absorption in the intestine of growing pigs. 8. Measurement of the flow of total lipid, acid-detergent fibre and volatile fatty acids. *British Journal of Nutrition* **42**, 279–287.
Samson, F. E., Dahl, N. & Dahl, D. R. (1956). A study on the narcotic actions of the short chain fatty acids. *Journal of Clinical Investigation* **35**, 1291–1298.
Scheppach, W., Fabian, C., Sachs, M. & Kasper, H. (1988). The effect of starch malabsorption on fecal short-chain fatty acid excretion in man. *Scandinavian Journal of Gastroenterology* **23**, 755–759.
Seal, C. J. & Mathers, J. C. (1989). Intestinal zinc transfer by everted gut sacs from rats given diets containing different amounts and types of dietary fibre. *British Journal of Nutrition* **62**, 151–163.
Selvendran, R. R. (1984). The plant cell wall as a source of dietary fiber: chemistry and structure. *American Journal of Clinical Nutrition* **39**, 320–337.
Silley, P. & Armstrong, D. G. (1984). Changes in metabolism of the rumen bacterium *Streptococcus bovis* H13/1 resulting from alteration in dilution rate and glucose supply per unit time. *Journal of Applied Bacteriology* **57**, 345–353.
Snoswell, A. M., Trimble, R. P., Fishlock, R. C., Storer, G. B. & Topping, D. L. (1982). Metabolic effects of acetate in perfused rat liver. Studies on ketogenesis, glucose output, lactate uptake and lipogenesis. *Biochimica et Biophysica Acta* **716**, 290–297.
Snow, P. & O'Dea, K. (1981). Factors affecting the rate of hydrolysis of starch in food. *American Journal of Clinical Nutrition* **34**, 2721–2727.
Stephen, A. M. & Cummings, J. H. (1980). The microbial contribution to human faecal mass. *Journal of Medical Microbiology* **13**, 45–56.
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.
Tulung, B., Rémésy, C. & Demigné, C. (1987). Specific effects of guar gum or gum arabic on adaptation of caecal digestion to high fibre diets in the rat. *Journal of Nutrition* **117**, 1556–1561.
Walter, D. J., Eastwood, M. A., Brydon, W. G. & Elton, R. A. (1988). Fermentation of wheat bran and gum arabic in rats fed on an elemental diet. *British Journal of Nutrition* **60**, 225–232.

Weaver, G. A., Krause, J. A., Miller, T. L. & Wolin, M. J. (1989). Constancy of glucose and starch fermentations by two different faecal microbial communities. *Gut* **30**, 19–25.
Williams, R. D. & Olmsted, W. H. (1936). The effect of cellulose, hemicellulose and lignin on the weight of the stool; contribution to the study of laxation in man. *Journal of Nutrition* **11**, 433–439.
Windmuller, H. G. & Spaeth, A. E. (1980). Respiratory fuels and nitrogen metabolism *in vivo* in small intestine of fed rats. *Journal of Biological Chemistry* **255**, 107–112.
Wolever, T. M. S., Cohen, Z., Thompson, L. U., Thorne, M. J., Jenkins, M. J. A., Prokipchuk, E. J. & Jenkins, D. J. A. (1986). Ileal loss of available carbohydrate in man: comparison of a breath hydrogen method with direct measurement using a human ileostomy model. *American Journal of Gastroenterology* **81**, 115–122.
Woodnutt, G. (1984). Absorption and utilization of metabolites in the rabbit. PhD Thesis, University of Reading.
Wyatt, G. M., Horn, N., Gee, J. M. & Johnson, I. T. (1988). Intestinal microflora and gastrointestinal adaptation in the rat in response to non-digestible dietary polysaccharides. *British Journal of Nutrition* **60**, 197–207.

Printed in Great Britain