

Digestion of complex carbohydrates and large bowel fermentation in rats fed on raw and cooked peas (*Pisum sativum*)

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Rats were fed on four semi-purified diets in which raw or cooked peas (*Pisum sativum* var. Sentinel) provided 250 or 500 g/kg diet. Pressure-cooking of peas followed by rapid freezing and freeze-drying increased the proportion of starch resistant to α -amylase (EC 3.2.1.1) without previous treatment with dimethyl sulphoxide. There were only minor effects of cooking on the digestibilities of non-starch polysaccharides and their constituent sugars. With the higher dietary pea concentration there were marked increases in the flow of organic matter to, and fermentation in, the large bowel. These increases were associated with significant increases in colonic tissue and contents weights, and in colonic transit time. Both cooking and dietary pea inclusion rate altered the pattern of volatile fatty acids in caecal contents and in portal blood.

Complex carbohydrates: Large bowel fermentation: Cooking: Peas

Leguminous seeds are normally cooked before being eaten. With beans, cooking improves nutritional value by destroying heat-labile anti-nutritional factors such as lectins (Liener & Kakade, 1980; Huisman *et al.* 1990). Peas (*Pisum sativum*) also contain lectins (Sharon & Halina, 1972), but these are present in very low concentrations (Grant *et al.* 1983; Goodlad, 1989) so that raw peas can be included in the diets of rats and pigs at up to 500 g/kg diet without adverse effects (Goodlad & Mathers, 1990, 1991). Although cooking is not essential for safe consumption of peas, thermal processing affects the carbohydrate fraction of the seed, in particular the starch. Mature peas contain approximately 470 g starch/kg dry matter (DM) (Paul & Southgate, 1978; Goodlad, 1989) of which almost one-third is as amylose (Orford *et al.* 1987; S. Cooper, R. M. Weightman & J. C. Mathers, unpublished results). Heating starch or starch-rich food in the presence of water results in gelatinization of the starch with preferential solubilization of amylose (Orford *et al.* 1987). This gelatinization increases the rate at which starch is hydrolysed by pancreatic α -amylase (EC 3.2.1.1) *in vitro* (Holm *et al.* 1988) and results in larger post-prandial concentrations of glucose and insulin in peripheral blood (Collings *et al.* 1981; O'Dea *et al.* 1981). On cooling to below 60°, starch undergoes retrogradation (a crystallization process; Collinson, 1968) leading to the formation of robust gels (Orford *et al.* 1987) in which some of the starch is resistant to α -amylase (RS; Englyst & Cummings, 1987). This appears to be mainly retrograded amylose and has been classified as RS₃ by Englyst & Kingman (1990). The extent of retrogradation depends on the starch source, with cereal starches retrograding more quickly than tuber starches because of their higher amylose content (Collinson, 1968; Orford *et al.* 1987). The conditions used for cooking and drying also affect the extent of RS

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formation (Englyst & Cummings, 1987). Effects of cooking on the non-starch polysaccharides (NSP) fraction in foods are poorly understood. For wheat flour, apparent increases in total dietary fibre with extrusion cooking seem to be due largely to the formation of RS (Björck *et al.* 1984). Baking and extrusion cooking of barley-based diets appear to increase the proportion of NSP which is soluble (Fadel *et al.* 1988, 1989), possibly as a result of depolymerization (Waldron & Selvendran, 1990).

Tissue softening and depolymerization of NSP following cooking (Waldron & Selvendran, 1990) may influence bacterial colonization and rate of fermentation within the large bowel (LB). In addition, alteration in the rate of starch digestion in the small bowel is likely to influence the nature and quantity of organic matter (OM) flowing into the LB with consequences for the amount of carbohydrate fermented in that organ. Changes in substrate supply probably alter the pattern of volatile fatty acids (VFA) produced (Macfarlane & Englyst, 1986; Cheng *et al.* 1987; Key & Mathers, 1987), and the amount of energy salvaged by the LB (Mathers, 1991), and may have other physiological effects (Cummings & Englyst, 1987; Wyatt *et al.* 1988). The present study was designed to compare the digestion of complex carbohydrates and large bowel fermentation in rats fed on raw and cooked peas, each provided at two dietary concentrations.

MATERIALS AND METHODS

Animals and housing

Thirty-two male Wistar rats (A. Tuck & Sons, Essex) with initial weight 134 (SE 2.2) g were randomly allocated in pairs to stock cages and each fed 20 g of the appropriate experimental diet (see Table 1) daily. After 10 d, four groups of seven rats 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

Four semi-purified diets were formulated (Table 1) to contain two concentrations (250 or 500 g/kg) of either milled cooked or raw peas (var. Sentinel), with balancing alterations in sucrose and casein to maintain similar crude protein (nitrogen $\times 6.25$) 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.

Whole dried peas were pressure-cooked in an equal volume of distilled water for 25 min. No water needed to be drained after cooking. Portions of the hot peas were immediately immersed in liquid N₂ for rapid freezing, followed by freeze-drying. Freeze-dried cooked peas and raw peas were hammer-milled to pass a 1 mm screen before incorporation into the diets. Each animal received 20 g air-dry ration at 10.00 hours daily. The ration was usually consumed by 13.00 hours. Any food residues were removed daily, dried and weighed. Water was available *ad lib*.

Experimental protocol

After 10 d in stock cages and 7 d in metabolism cages there followed a 7 d balance period with total collection of faeces and urine, and measurement of food intake. The appropriate experimental diets were fed throughout the whole of this 24 d study. Animals were weighed at the beginning and end of the balance period, and on the days of slaughter.

Animals were anaesthetized and blood, digesta and tissue samples were collected as described by Goodlad & Mathers (1990). In addition, the masses of colon and of colonic tissue were measured. Fourteen animals were sampled on each of two consecutive days.

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

Diet ...	LC	HC	LR	HR
Raw peas (<i>Pisum sativum</i>)*	0	0	250	500
Cooked peas†	250	500	0	0
Maize starch‡	398	398	398	398
Sucrose	190	0.6	190	0.6
Casein	95	33	95	33
Maize oil	33	33	33	33
Vitamin and mineral mix§	31.7	31.7	31.7	31.7
L-methionine	2.3	3.9	2.3	3.9
Analysed composition (g/kg DM)				
Organic matter	967	959	958	947
Crude protein (nitrogen × 6.25)	144	150	149	149
Total NSP	25.9	63.3	29.3	64.7
Cellulose	9.8	25.0	12.6	28.7
NCP	16.7	38.3	16.7	36.0
Arabinose	6.3	15.1	7.5	16.2
Xylose	1.5	4.6	2.0	6.1
Galactose	1.1	3.3	1.4	3.2
Glucose	16.9	38.7	18.5	35.8
Uronic acids	0.03	0.07	0.04	0.08
Resistant starch	7.6	14.5	3.9	3.6
Calculated energy content (MJ ME/kg)	14.4	14.0	14.4	14.0

LC, HC, LR, HR low (L) and high (H) levels of cooked (C) and raw (R) peas; NSP, non-starch polysaccharide; NCP, non-cellulosic polysaccharide; ME, metabolizable energy.

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

† Pressure cooked for 25 min (for details, see p. 476).

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

§ For details, see Goodlad & Mathers (1990).

Analytical methods

NSP and its constituents were determined as described by Englyst & Cummings (1984) and RS by omitting the dimethyl sulphoxide (DMSO) addition step. N in diets, faeces and urine was measured by a Kjeldahl procedure, Cr₂O₃ and volatile fatty acids (VFA) in caecal digesta as described by Mathers *et al.* (1990), organic matter (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).

Statistical methods

Data were examined by one-way analysis of variance with orthogonal contrasts (Ridgman, 1975) used to test: (1) effects of dietary concentration of peas, i.e. contrast 1 was (low, cooked (LC)+low, raw (LR)) *v.* (high, cooked (HC)+high, raw (HR)), (2) effects of cooking, i.e. contrast 2 was (LC+HC) *v.* (LR+HR) and (3) interactions between dietary concentration and cooking, i.e. contrast 3 was (LC+HR) *v.* (HC+LR). Results are presented as means for each diet (*n* 7) with their standard errors based on between-animals within-diets variation.

Table 2. *Dry matter (DM) intake (g/7 d), growth rate (g/7 d), liver weight (g) and aspects of nitrogen metabolism (g/7 d) in rats given diets containing raw (R) or cooked (C) peas (Pisum sativum var. Sentinel) at two inclusion rates (250 (L) and 500 (H) g/kg)*

Diet†...	(Means for seven rats per diet)					Statistical significance of dietary effects‡		
	LC	HC	LR	HR	SEM	Inclusion rate	Cooking	Interaction
	DM intake	127	121	126	123	0.9	***	NS
Growth rate	39.2	40.6	44.1	39.9	1.12	NS	NS	*
Liver wt	11.0	9.2	10.9	9.9	0.26	***	NS	NS
N metabolism								
N intake	2.93	2.91	3.01	2.95	0.023	NS	*	NS
Faecal N	0.36	0.52	0.39	0.51	0.016	***	NS	NS
Urinary N	0.88	0.81	0.90	0.81	0.065	NS	NS	NS
N retention	1.69	1.59	1.72	1.63	0.056	NS	NS	NS

NS, not significant.

* $P < 0.05$; *** $P < 0.001$.

† For details of composition see Table 1.

‡ For details of orthogonal contrasts see p. 477.

RESULTS

The most striking effect of cooking of peas on diet composition was the 2–4-fold increase in the concentration of starch resistant to pancreatic α -amylase without previous treatment with DMSO (RS; Table 1). Concentrations of NSP and of its constituent sugars were similar for raw- and cooked-pea diets and increased approximately 2-fold when pea inclusion rate increased from 250 to 500 g/kg diet. The lower-than-expected NSP concentration for diet LC was due largely to the apparently lower cellulose content of this diet.

Rat growth and N metabolism

All diets were well-accepted by the rats; food refusals were negligible and growth rates good (Table 2). The statistically significant ($P < 0.05$) interaction between pea inclusion rate and cooking was due to a 10% higher growth rate on the LR diet whilst growth rates for the other three diets were very similar. Higher intakes of peas were associated with higher faecal N outputs ($P < 0.01$), but these were balanced by a tendency (not significant) towards reduced urinary N outputs so that N retention was not statistically different between diets.

Sites and extents of DM and OM digestion

Cooking of peas had no significant effects on the extent of digestion of dietary DM in the upper or the lower intestine. However, increasing the proportion of peas from 250 to 500 g/kg diet caused a large fall in DM digestibility anterior to the caecum and, although this was compensated for by increased LB digestion, whole gut digestibility of DM was significantly reduced ($P < 0.001$). Estimates of DM digestibility measured at the colon and in faeces were similar. Mouth-to-anus digestibilities of DM were calculated from total DM intakes and faecal outputs during the 7 d balance period and by the marker ratio technique, with both methods giving similar results (Table 3). Faecal recovery of the indigestible flow marker Cr_2O_3 was 1.07 (SE 0.012).

Table 3. Apparent dry matter (DM) digestibility† measured at various sampling sites in rats given diets containing raw (R) or cooked (C) peas (*Pisum sativum* var. *Sentinel*) at two inclusion rates (250 (L) and 500 (H) g/kg)

(Means for seven rats per diet)

Diet‡...	LC	HC	LR	HR	SEM	Statistical significance of dietary effects§		
						Inclusion rate	Cooking	Interaction
Terminal ileum	0.81	0.74	0.81	0.73	0.018	***	NS	NS
Colon	0.95	0.93	0.95	0.93	0.003	***	NS	NS
Faeces	0.94	0.91	0.94	0.91	0.002	***	NS	NS
Faeces (method 1)	0.94	0.91	0.94	0.91	0.002	***	NS	NS

NS, not significant.

*** $P < 0.001$.

† Digestibilities were determined by the marker ratio method except for faeces (method 1) calculated on the basis of total faecal collection.

‡ For details of composition, see Table 1.

§ For details of orthogonal contrasts, see p. 477.

With the higher dietary concentration of peas, flows of DM and of OM to the LB were significantly increased and were accompanied by mean increased net daily disappearances of 0.68 g DM and 0.92 g OM, so that OM disappearance in the LB with diets HC and HR was almost twice as great as with the LC and LR diets (Table 4).

Mass of LB tissue and digesta

Weights of whole caecum, caecal tissue and wet caecal contents tended to increase with the higher intakes of peas but the differences were not significant (Table 5). Caecal digesta DM was significantly ($P < 0.05$) increased with diets HC and HR, when compared with LC and LR, due to the combination of slightly higher wet contents weight and slightly higher proportion of DM in the digesta. Caecal transit time (TT) was about one-third shorter for the HR than for the LR diet with both cooked-pea diets (LC and HC) giving intermediate and more similar TT (Table 5). For the colon, the results were much clearer with the higher-pea diets (HC and HR) resulting in significantly greater weights for the whole colon, colonic tissue and colonic wet digesta and DM contents and significantly ($P < 0.05$) longer TT. Cooking peas had no significant effect on any of these variables.

Caecal pH and fermentation pattern

Caecal pH was significantly reduced by consumption of the higher-pea diets whilst total VFA concentration increased (Table 6). The latter was also markedly higher for the cooked-pea-containing diets than for the raw-pea-containing diets. The proportion of peas in the diet had no significant effects on the molar proportions of the three major VFA (acetate, propionate and butyrate), but cooking was associated with significantly higher molar proportions of acetate and lower proportions of propionate. Molar proportions of isobutyrate, isovalerate and valerate were lower for the cooked-pea diets than for the raw-pea diets and were reduced with the higher dietary pea inclusion rate. Hexanoate was detected in caecal contents of rats on all diets with no significant between-diets effects.

Table 4. *Flows (g/d) of dry matter and organic matter to, and disappearance in, the large bowel (LB) of rats given diets containing raw (R) or cooked (C) peas (Pisum sativum var. Sentinel) at two inclusion rates (250 (L) and 500 (H) g/kg)*

(Means for seven rats per diet)

Diet†...	LC	HC	LR	HR	SEM	Statistical significance of dietary effects‡		
						Inclusion rate	Cooking	Interaction
Dry matter								
Ileal flow	3.38	4.48	3.43	4.73	0.334	**	NS	NS
Faecal output	1.09	1.63	1.11	1.61	0.033	***	NS	NS
LB disappearance	2.29	2.86	2.33	3.12	0.321	*	NS	NS
Organic matter								
Ileal flow	1.91	2.99	1.56	3.31	0.172	***	NS	NS
Faecal output	0.70	1.17	0.76	1.27	0.038	***	NS	NS
LB disappearance	1.21	1.82	0.80	2.03	0.160	***	NS	NS

NS, not significant.

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

† For details of composition, see Table 1.

‡ For details of orthogonal contrasts, see p. 477.

Table 5. *Large bowel tissue and digesta weights (g) and transit times (d) in rats given diets containing raw (R) or cooked (C) peas (Pisum sativum var. Sentinel) at two inclusion rates (250 (L) and 500 (H) g/kg)*

(Means for seven rats per diet)

Diet†...	LC	HC	LR	HR	SEM	Statistical significance of dietary effects‡		
						Inclusion rate	Cooking	Interaction
Caecum								
Organ mass	3.45	4.65	3.79	2.82	0.303	NS	NS	NS
Wet digesta	2.65	3.68	2.91	2.95	0.266	NS	NS	NS
Digesta DM	0.44	0.64	0.49	0.54	0.046	*	NS	NS
Tissue	0.79	0.96	0.87	0.86	0.052	NS	NS	NS
Transit time	0.42	0.49	0.57	0.39	0.039	NS	NS	**
Colon								
Organ mass	1.82	2.63	1.77	2.86	0.156	***	NS	NS
Wet digesta	0.81	1.49	0.75	1.73	0.149	***	NS	NS
Digesta DM	0.27	0.48	0.24	0.58	0.062	***	NS	NS
Tissue	1.01	1.14	1.01	1.13	0.041	**	NS	NS
Transit time	0.27	0.41	0.29	0.45	0.057	*	NS	NS

NS, not significant; DM, dry matter.

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

† For details of composition, see Table 1.

‡ For details of orthogonal contrasts, see p. 477.

Table 6. *pH, total volatile fatty acids (VFA) concentration (mmol/kg wet caecal contents) and molar proportions of the individual VFA in the caeca of rats given diets containing raw (R) or cooked (C) peas (Pisum sativum var. Sentinel) at two inclusion rates (250 (L) and 500 (H) g/kg)*

(Means for seven rats per diet)

Diet†...	LC	HC	LR	HR	SEM	Statistical significance of dietary effects‡		
						Inclusion rate	Cooking	Interaction
Caecal pH	6.34	6.16	6.44	6.18	0.057	**	NS	NS
Total VFA (mmol/kg wet caecal contents)	140	186	115	131	7.3	***	***	NS
Molar proportions of individual VFA (mmol/mol)								
Acetate	649	678	619	615	9.3	NS	***	NS
Propionate	155	142	177	139	5.8	NS	***	NS
Isobutyrate	5	3	8	6	0.7	*	***	NS
Butyrate	155	154	154	198	11.9	NS	NS	NS
Isovalerate	8	3	14	10	1.2	**	***	NS
Valerate	14	9	16	14	1.4	*	*	NS
Hexanoate	13	10	12	17	2.4	NS	NS	NS

NS, not significant.

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

† For details of composition, see Table 1.

‡ For details of orthogonal contrasts, see p. 477.

Table 7. *Apparent digestibilities of non-starch polysaccharides (NSP) fraction and its constituents, and of resistant starch (RS) in rats given diets containing raw (R) or cooked (C) peas (Pisum sativum var. Sentinel) at two inclusion rates (250 (L) and 500 (H) g/kg)*

(Means for seven rats per diet)

Diet†...	LC	HC	LR	HR	SEM	Statistical significance of dietary effects‡		
						Inclusion rate	Cooking	Interaction
NSP	0.56	0.63	0.55	0.62	0.030	*	NS	NS
Cellulose	0.14	0.38	0.33	0.43	0.061	*	NS	NS
NCP	0.79	0.79	0.72	0.77	0.014	NS	*	NS
Arabinose	0.98	0.97	0.96	0.97	0.004	NS	*	NS
Xylose	0.16	0.37	0.21	0.49	0.062	***	NS	NS
Galactose	0.63	0.75	0.57	0.72	0.026	***	NS	NS
Glucose	0.48	0.58	0.49	0.51	0.202	NS	*	NS
Uronic acids	0.89	0.89	0.83	0.88	0.014	NS	*	NS
RS	0.99	1.05	0.99	1.04	0.093	NS	NS	NS

NCP, non-cellulosic polysaccharides; NS, not significant.

* $P < 0.05$; *** $P < 0.001$.

† For details of composition, see Table 1.

‡ For details of orthogonal contrasts, see p. 477.

Digestibility of NSP and RS

Digestibilities of total NSP were very similar for raw-pea diets and for cooked-pea diets and were greater for the diets containing 500 g peas compared with those containing 250 g peas/kg diet (Table 7). Cellulose digestibility was lower than for total NSP and increased with the higher pea inclusion rate. The particularly low value recorded for cellulose digestibility with diet LC may be an artifact of the lower-than-expected analytical value for the cellulose content of this diet (Table 1). Digestibilities of the total non-cellulosic polysaccharides (NCP) fraction and of its monomers, arabinose and uronic acids, were significantly ($P < 0.05$) higher for the cooked-pea diets and unaffected by pea inclusion rate. In contrast, digestibilities of xylose and galactose were much higher for diets HC and HR than for diets LC and LR. Overall, digestibilities of NCP sugars were in the order arabinose > uronic acids > galactose > xylose. Digestibility of RS was not significantly different from 1.0 for any diet.

Blood concentration of VFA and 3OHB

Portal blood contained measurable concentrations of all those major VFA found in caecal contents and concentrations were increased with the higher-pea diets (Table 8). Cooking of peas before inclusion into the diet resulted in significantly ($P < 0.01$) lower concentrations of acetate in portal blood but had no other effects on the measured blood metabolites. Acetate was the only VFA detected reliably in heart blood and this too was higher with diets HC and HR than with diets LC and LR. Concentrations of 3OHB in both portal and heart blood tended to increase with the higher-pea diets but the difference was not statistically significant.

DISCUSSION

Effects of cooking on dietary starch

When compared with cereals, the rate of digestion of starch from leguminous seeds including peas is slow (Gee & Johnson, 1985; Würsch *et al.* 1986) and this is reflected in reduced concentrations of glucose and insulin post prandially (Thorne *et al.* 1985; Tappy *et al.* 1986). This slow rate of starch digestion in raw peas is unlikely to be caused by α -amylase inhibitors, since these are barely detectable (Goodlad, 1989), and appears to be due to the physical entrapping of starch within fibrous thick-walled parenchyma cells (Würsch *et al.* 1986). Starch in this form has been referred to as physically inaccessible starch (RS₁) by Englyst & Kingman (1990). Cooking in water swells these starch granules and gelatinizes the starch and, especially when the cells are broken, increases starch digestion rate (Würsch *et al.* 1986). Our cooked-pea-containing diet had higher concentrations of starch resistant to α -amylase without previous treatment with DMSO (RS₃) (Table 1) so that it would appear that whilst cooking may have increased the potential rate of starch digestion, the subsequent cooling, freezing and freeze-drying more than balanced this through the formation of retrograded amylose (RS₃; Englyst & Kingman, 1990). Bach Knudsen *et al.* (1988) reported that cooking and subsequent cooling with or without freeze-drying greatly increased the concentration of RS in sorghum (*Sorghum bicolor* (L.) Moench).

Food intake, growth and body composition

Diets containing raw and cooked peas were well accepted by the rats. The lower DM intakes observed with the higher pea inclusion rate is a consequence of the slightly lower DM content of these diets. The statistically significant ($P < 0.05$) interaction between inclusion rate and cooking on growth rate is due to the 10% greater growth rate by animals given diet LR with very similar growth rates for the other three diets. We have no

Table 8. Concentrations of volatile fatty acids (VFA; μM) and of 3-hydroxybutyrate (3OHB; μM) in whole blood from rats given diets containing raw (R) or cooked (C) peas (*Pisum sativum* var. *Sentinel*) at two inclusion rates (250 (L) and 500 (H) g/kg)

(Means for seven rats per diet)

Diet†...	LC	HC	LR	HR	SEM	Statistical significance of dietary effects‡		
						Inclusion rate	Cooking	Interaction
Portal blood								
Acetate	690	709	754	968	52.1	*	**	NS
Propionate	103	156	104	174	12.6	***	NS	NS
Butyrate	59	88	49	103	8.6	***	NS	NS
3OHB	53	54	57	69	12.1	NS	NS	NS
Heart blood								
Acetate	596	693	529	620	41.3	*	NS	NS
3OHB	5	90	89	94	12.3	NS	NS	NS

NS, not significant.

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

† For details of composition, see Table 1.

‡ For details of orthogonal contrasts, see p. 477.

satisfactory explanation for this observation, although it is worth noting that diet LR produced the greatest N retention so the effect may be real. It appeared that diet had no marked effect on the composition (fat:lean ratio) of live-weight gain since the ratio of weight gain:N retention was similar for all diets. The very highly significantly lower liver weights with the high-pea diets may indicate lower contents of glycogen and lipid (Pedroso *et al.* 1990).

Sites and extents of digestion

We were unable to detect any effect of cooking on the flow of OM from the terminal ileum or on the amount of OM apparently fermented in the LB (Table 4). Since the OM flowing into the LB was not chemically fractionated, we do not know whether cooking altered the composition of this OM, e.g. exchanging RS_1 for RS_3 . With 500 v. 250 g peas/kg diet included at the expense of the highly digestible sucrose and casein, there were significant increases in the flows of DM and OM from the terminal ileum (Table 4). As a proportion of ingested OM, ileal OM flow increased from 0.10 to 0.19 with 250 v. 500 g peas/kg diets. If it is assumed that all the ingested NSP and RS escaped digestion in the small intestine (Englyst & Cummings, 1985), then it may be calculated that these fractions accounted for 0.35 (low-pea diets) and 0.41 (high-pea diets) of the ileal OM flow. This suggests that there are substantial amounts of other substances probably including oligosaccharides, proteins and starch of food origin and endogenous materials contributing to LB substrate supply (Cummings & Englyst, 1987).

Much of the OM entering the caecum (0.57 and 0.61 for low- and high-pea diets respectively) did not appear in the faeces (Table 5), and it is assumed that this material was fermented largely within the caecum since digestibilities measured at the level of the colon were similar to those for faeces (Table 3). True digestibility of OM within the LB will have been greater than this since some of the fermented OM will have been used for synthesis of bacterial cells. The latter is estimated to be 263 mg bacterial OM/g OM truly fermented

(Mathers, 1991) from values given by Cummings (1984). The greater faecal N output with the higher-pea diets (Table 2) is consistent with increased bacterial cell output (Goodlad & Mathers, 1990) as a result of the greater OM fermentation with these diets. In sheep, starch infused into the caecum was extensively fermented and increased faecal N output (Ørskov *et al.* 1970).

Whilst cooking may influence the degree of polymerization of NSP in foods (Waldron & Selvendran, 1990), the limited information available (Björck *et al.* 1984; Fadel *et al.* 1988, 1989) suggest that thermal processing (baking or extrusion cooking) of cereals has only small effects on NSP digestibility. This was confirmed in the present study where digestibilities of total NSP from raw and boiled peas were not significantly different. Whilst cooking did tend to increase the digestibilities of NCP and its constituent sugars, perhaps through disruption of plant cell walls and partial hydrolysis of their polymeric constituents (Waldron & Selvendran, 1990), the nutritional consequences of such small changes are likely to be minor. NSP digestibility was lower for cooked than for raw peas when tubed to cockerels (Longstaff & McNab, 1987).

In the earlier studies (Goodlad & Mathers, 1990, 1991; Key & Mathers, 1990) it was reported that variations in the dietary intake of NSP from raw peas or cooked haricot beans (*Phaseolus vulgaris*) were matched by proportional changes in faecal NSP output in both rats and pigs, i.e. that each increment in NSP intake resulted in equally effective NSP digestion. The significantly higher apparent digestibilities of NSP and some of the monomeric constituents, notably xylose and galactose with its higher dietary pea inclusion rate (Table 7) in the present experiment were, therefore, unexpected, and indicated that it may not always be safe to assume that digestibility of the NSP in a food is independent of intake. Apparent digestibilities of NSP and of its constituents in the peas (var. Sentinel) used in the present study were lower than for those (var. Progreta) reported earlier (Goodlad & Mathers, 1990). The total NSP and their monosaccharide constituents were similar for both varieties (Goodlad, 1989) so cannot explain this apparent between-variety difference in digestibility.

RS prepared from pea starch by autoclaving then cooling to 1° for 72 h was more slowly fermented *in vitro* in dilute incubations with caecal contents from rats or fresh human faeces than was maize RS prepared in the same way (Wyatt & Horn, 1988). *In vivo* evaluation of similar products indicated that pea RS was relatively poorly digested by the rat in the first few days after introduction to the diet, but by 17–18 d the difference in apparent digestibility between the two sources of RS was reduced and both were ≥ 0.9 (Faulks *et al.* 1989). We could not detect consistently any RS in the faeces from our rats and assume that it was virtually all fermented.

LB fermentation

Feeding cooked *v.* raw peas had no significant effects on caecal or colonic tissue or contents weights. However, rats fed on diets with the higher inclusion rate of peas had greater LB digesta weights, especially for the colon, and also much longer colonic TT (Table 5). These findings suggest that with the higher intakes of fermentable material some spill-over of fermentation into the colon may have occurred and the longer LB TT may have contributed to the higher NSP digestibilities recorded with these diets.

The mass of digesta in the caecum and colon was positively associated with tissue weight for both organs, as described previously by Wyatt *et al.* (1988) and Goodlad & Mathers (1990) (caecum only). Since the accumulation of digesta in the caecum was independent of the flow-rate of DM or OM into the organ or of the amount of OM fermented in the LB, in agreement with the observations by Mathers & Dawson (1991), the factors influencing LB hypertrophy remain unclear.

Table 9. Calculated absorption† of volatile fatty acids (VFA; mmol/d) from the large bowel and its contribution to metabolizable energy (ME) intake in rats given diets containing raw (R) or cooked (C) peas (*Pisum sativum* var. *Sentinel*) at two inclusion rates (250 (L) and 500 (H) g/kg)

(Means for seven rats per diet)

Diet†...	LC	HC	LR	HR	SEM	Statistical significance of dietary effects§		
						Inclusion rate	Cooking	Interaction
Acetate	8.8	13.1	5.5	13.4	1.22	***	NS	NS
Propionate	2.1	2.7	1.5	2.8	0.30	***	NS	NS
Butyrate	2.0	3.3	1.4	4.4	0.35	***	NS	NS
Total	12.9	19.2	8.4	20.7	1.72	***	NS	NS
Proportion of ME intake	0.05	0.08	0.04	0.09	0.007	***	NS	NS

NS, not significant.

*** $P < 0.001$.

† Calculated from organic matter disappearance in the large bowel (Table 4) and caecal VFA molar proportions (Table 6) assuming conventional anaerobic stoichiometry (Demeyer & Van Nevel, 1975).

‡ For details of composition, see Table 1.

§ For details of orthogonal contrasts, see p. 477.

Higher intakes of peas resulted in lower caecal pH and higher total VFA concentrations as expected from the greater daily rate of OM fermentation in that organ. The higher total VFA concentration with cooked than with raw peas may indicate a faster rate of OM fermentation occurring at the time of sampling caused not by greater OM flow from the terminal ileum (Table 4) but possibly by more ready access to cell-wall polysaccharides. Although cooking has only minor effects on the extent of NSP fermentation (Table 7), it may alter the rate and this deserves further investigation.

Changes in VFA molar proportions with alterations in diet composition were not as marked as in some earlier studies (Key & Mathers, 1987, 1988; Mathers *et al.* 1990; Goodlad & Mathers, 1990) possibly because of smaller contrasts in the amount and nature of substrates supplied to the LB. The reason for the higher acetate molar proportion with the cooked-pea diets is unknown. We have observed relatively high acetate concentrations both in vitro (Goodlad & Mathers, 1988) and in vivo (Mathers & Finlayson, 1989) with pectin as substrate. The latter is rapidly fermented so it is possible that differences in rate of fermentation could have contributed to the altered VFA pattern observed between raw- and cooked-pea diets. The reduced proportions of branched-chain VFA and of valerate with the cooked-pea diets could indicate their greater utilization for *de novo* amino acid and, hence, bacterial protein synthesis (Russell & Hespell, 1981). Alternative possibilities include either less protein flowing from the terminal ileum or that the heat-treated dietary protein escaping small intestinal digestion was also less susceptible to LB fermentation.

Absorption of fermentation end-products

Amounts of VFA calculated to be absorbed from the LB (Table 9) were almost doubled by the doubling of pea-inclusion rate in the diets with no significant effects of cooking. These increased absorption rates were reflected in higher concentrations of VFA in portal

and, for acetate only, in heart blood. The significantly higher concentrations of acetate in portal blood with the raw-pea diets may indicate that, at the time of blood sampling (4–7 h after presentation of the last meal), the instantaneous rate of LB fermentation was higher for animals consuming raw peas compared with cooked peas. In agreement with results from our earlier study (Goodlad & Mathers, 1990), the rat liver removed all detectable propionate and butyrate but only a portion of the acetate supplied by the LB via the portal vein, so that direct effects of substrate supply on metabolism is anticipated for both liver (Pedroso *et al.* 1990) and extrahepatic tissues.

In conclusion, cooking peas had only minor effects on the extent of digestion of NSP but may have altered the rate and sites of starch digestion, as evidenced by caecal fermentation changes. Fractionation of the OM flowing into the LB and detailed study of the rates of hydrolysis of individual complex carbohydrates by the LB flora may aid understanding of the effects of culinary processes on LB fermentation and subsequent implications for tissue metabolism and health.

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