Effect of baked beans (*Phaseolus vulgaris*) on steroid metabolism and non-starch polysaccharide output of hypercholesterolaemic pigs with or without an ileo-rectal anastomosis

BY NEUZA M. B. COSTA[1], A. GRAHAM LOW[1], ANN F. WALKER[1]*, ROBERT W. OWEN[2] AND HANS N. ENGLYST[3]

[1] *Department of Food Science and Technology, University of Reading, Whiteknights, Reading RG6 2AP*

[2] *PHLS Centre for Applied Microbiology and Research, Division of Biotechnology, Sensor Development Group, Porton Down, Salisbury SP4 0JG*

[3] *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

(*Received 12 February 1993 – Revised 9 September 1993 – Accepted 23 September 1993*)

The plasma-cholesterol-lowering effects of some dietary legumes are now well established from animal and human studies, but the mechanism is not completely understood. The present study investigated the effect of baked beans (*Phaseolus vulgaris*) on steroid metabolism of hypercholesterolaemic pigs. Three groups of four pigs were studied: baseline (BL), normal pigs (NP) and those previously prepared with an ileo-rectal anastomosis to nullify the function of the large intestine (IR). All three groups were given a semi-purified control diet, with about 40% energy as fat (polyunsaturated: saturated fatty acid (P:S) ratio 0·3), supplemented with 10 g cholesterol/kg, for 14 d. Then IR and NP pigs were fed for 28 d on a diet supplemented with 10 g cholesterol/kg and 300 g baked beans/kg (dry-matter basis), so that the 40% contribution to energy from fat was maintained (P:S ratio 0·3). Group BL was fed on the control diet throughout. The intact pigs (NP) fed on baked beans showed considerable differences compared with the other groups, as follows: (*a*) reduced plasma cholesterol (NS); (*b*) higher concentration of cholesterol in bile (NS); (*c*) higher concentration of bile acids, especially secondary bile acids, in bile ($P < 0·05$); (*d*) reduced elimination of bile acids in faeces, especially secondary bile acids ($P < 0·05$); (*e*) higher excretion of coprostanol and lower elimination of cholesterol in faeces ($P < 0·05$). From these findings it is proposed that a baked-bean-enriched diet potentiates bacterial fermentation and steroid degradation in the large intestine and enhances conservation of bile acids and cholesterol within the enterohepatic circulation. The high concentration of bile acids and cholesterol in bile may thus promote feedback inhibition of hepatic cholesterol synthesis, and hence, reduce plasma cholesterol.

Baked beans: Plasma cholesterol: Steroids: Ileo-rectal anastomosis: Pig

Considerable evidence has accumulated in recent years regarding the cholesterol-lowering effect of legumes (Shutler *et al.* 1987*a*, *b*; Kingman, 1991). The basis of this effect is not completely understood, although some hypotheses have proposed that particular components, such as dietary fibre (Anderson & Chen, 1979) and saponins (Oakenfull *et al.* 1979; Sidhu *et al.* 1987), are responsible factors. The effect of these components in enhancing the faecal excretion of cholesterol and bile-acid derivatives has been put forward as one explanation, but the evidence for this is controversial. Previous findings from this laboratory have shown that baked beans (*Phaseolus vulgaris*) reduce plasma cholesterol when fed to hypercholesterolaemic pigs at a level of 300 g/kg, on a dry-matter basis

* For reprints.

(Shutler *et al.* 1988; Costa *et al.* 1993). In the experiment carried out by Costa *et al.* (1993), a significant reduction was observed in the cholesterol deposition in the pig liver. These findings suggest that the consumption of baked beans may have increased the cholesterol excretion and/or the bile-acid pool size and turnover. Since the excretion of bile acids in the faeces is a principal pathway for the removal of cholesterol from the body, the measurement of faecal steroids is central to understanding the mechanism of the cholesterol-lowering effect of baked beans. Measurement of the digesta before its entry into the large intestine would facilitate quantification of primary bile acid production and absorption. An estimate of cholesterol absorption and its recycling via bile, before modification by the intestinal flora, can be obtained using pigs with an ileo-rectal anastomosis (IR).

The present study aimed to investigate the effect of baked beans on the enterohepatic circulation of cholesterol and bile acids and the influence of the large intestine on this process. For this purpose comparisons between the steroid profile of bile and the outputs of faecal steroids of hypercholesterolaemic pigs with and without IR were carried out.

MATERIALS AND METHODS

Animals

Twelve Large White × Landrace male pigs of about 35 kg live weight were equally divided into three experimental groups, baseline (BL), normal pigs (NP) and ileo-rectal anastomosis (IR). Pigs of the IR group were surgically prepared with an ileo-rectal anastomosis with complete isolation of the large intestine, as described below. This group was kept in metabolism cages throughout the experiment to facilitate sampling and animal care. The other pigs were maintained in individual floor pens and transferred to metabolism cages during the faecal collection periods. Pens and cages were located in the same temperature-controlled room ($23 \pm 3°$). The animals were weighed at weekly intervals in order to monitor growth and calculate feed requirement.

Ileo-rectal anastomosis

Pigs were deprived of food for 18 h and, immediately before surgery, they were tranquilized with an intra-muscular injection of 5 ml Ketamine. General anaesthesia was induced with 10 ml Thiopentone (50 g/l, intra-muscular) and maintained with a 1–2% fluothane in oxygen mixture throughout the operation. The surgical area was cleaned, shaved and disinfected by scrubbing with an iodine solution. Surgery was performed as described by Green *et al.* (1987). The process started with a longitudinal 150 mm incision in the abdomen. Then the terminal ileum was transected and the distal cut end was closed. The descending distal colon was exteriorized and a longitudinal opening made at approximately 120 mm from the anus. The open end of the ileum was positioned over the incision in the colon and sutured, forming a T-shaped ileum–colon junction. Although the large gut was not removed, the lumen of the colon was completely isolated by suture from the rest of alimentary tract. After surgery, pigs were fasted for 18 h and then encouraged to eat at regular intervals small amounts of a commercial diet mixed with water. The antibiotic ampicillin (150 mg, Penbritin) was administered on the day of the surgery and repeated for the following 3 d.

Diets

An attempt was made to use only pigs which were hyper-responsive to cholesterol-feeding in the experimental groups. For this reason, six pigs were prepared surgically and, together with eleven intact pigs, were fed on a hypercholesterolaemic control diet (diet C) for 14 d.

After this period the four anastomosed pigs with the highest levels of plasma cholesterol were selected for group IR. In the same way, the eight intact (or normal) pigs with the highest cholesterol levels were selected and randomly divided into two equal groups (BL and NP), in such a way that the mean values of plasma cholesterol between groups were kept as close as possible. Group BL was kept on diet C, and groups NP and IR were placed on a slightly modified diet (diet B3), substituted with baked beans at a level of 300 g/kg, on a dry-matter basis, for a further 28 d. The compositions of diets C and B3 are given in Table 1. All diets provided about 12% energy as protein, 40% as fat and 48% as carbohydrate, with a polyunsaturated:saturated fatty acids (P:S) ratio of about 0·3. All the diets were fed at a level of 30 g/kg body weight per d in two equal meals, at 08.00 and 15.30 hours. Tap water (2·5 l/kg dry weight) was added to the diet immediately before feeding. The amount of water given to the pigs receiving baked beans was altered to account for the moisture content of the beans. Additional water was available *ad lib.* between meals. IR pigs received a supplement of $NaHCO_3$ (5 g/l) and NaCl (5 g/l) mixed in additional water, to compensate for the loss of electrolytes due to the gut bypass.

Blood sampling

Fasting blood samples (about 10 ml) were taken by venepuncture into heparinized tubes at fortnightly intervals. Plasma was separated by centrifugation at 1500 ***g*** for 15 min and stored at −18° for estimation of circulating metabolites. Analyses of total cholesterol (Allain *et al.* 1974), triacylglycerol (Fossati & Prencipe, 1982) and high-density-lipoprotein (HDL)-cholesterol (Warnick *et al.* 1982) were carried out.

Bile collection

The intact gall-bladder was taken immediately after slaughter of the animals and was stored at −18° for analysis of the biliary steroid profile. After thawing, bile samples were deproteinated by mixing 1 ml bile with 20 ml ethanol, incubating overnight at 37° and spinning at 2000 ***g*** for 20 min. The supernatant was taken and the lower phase was mixed with another 20 ml ethanol and spun twice subsequently, as described by Houghton *et al.* (1989). The pooled supernatants, containing the deproteinated bile, were evaporated on a heating block to dryness and kept for analysis of conjugated bile acids and cholesterol.

The biliary conjugated bile acids were analysed by HPLC, as described by Spigelman *et al.* (1991). Analyses were carried out with a Waters HPLC chromatograph (Waters Ass., Milford, MA, USA), fitted with a Nova-Pak reverse-phase cartridge column (100 mm × 8 mm i.d.) compressed in a Waters RCM-100 module. The mobile phase was prepared with 150 ml methanol (HPLC grade) + 50 ml 0·01 M-KH_2PO_4 + 0·41 ml 10 M-NaOH, and the pH was adjusted to 5·5 by the addition of H_3PO_4 (850 ml/l). The mobile phase was filtered and degassed with He before use. A flow rate of 0·7 ml/min was used throughout the analysis. Detection was by a variable wavelength Lamda-Max 481 LC spectrophotometer set at 205 nm.

The dry deproteinated bile samples were resuspended with 10 ml mobile phase, containing 5 mg/ml glycodeoxycholic acid as internal standard. Samples were vortex-mixed and filtered into small vials. The conjugated bile acids were identified by reference to known standards and were quantified relative to the internal standard, using a Waters-740 data module as an integrator.

The biliary cholesterol was determined by GLC on a Pye Unicam series 304 chromatograph. A silanized glass column (2 m × 2 mm i.d.) was packed with Supelcoport (80–100 mesh), coated with 3% OV-1 (Superchem Ltd, Saffron Walden, Essex). The carrier

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

Ingredient	Diet	
	Control (C)	Bean (B3)
Baked beans (*Phaseolus vulgaris*) (dry wt)	—	300·0
Maize starch	477·9	357·8
Sucrose	84·0	61·8
Soya-bean oil	30·0	25·0
Silkido (beef tallow)	145·0	145·0
Casein	157·0	76·0
Solka-floc (cellulose)	57·0	—
Mineral mix*	10·0	7·0
Vitamin mix†	2·0	1·4
Dicalcium phosphate	31·0	21·7
Choline chloride	1·1	0·8
Sodium chloride	5·0	3·5
Total	1000·0	1000·0
Cholesterol	10·0	10·0

* The mineral mix comprised (g/kg): K_2CO_3 447·0, $MgCO_3.3\,H_2O$ 173·0, $FeSO_4.7\,H_2O$ 33·0, $ZnCO_3$ 10·0, $MnSO_4.4\,H_2O$ 8·0, $CuSO_4.5\,H_2O$ 1·7, NaF 0·8, $CoCl_2$ 0·6, maize starch 325·8.

† The vitamin mix comprised (g/kg): retinol 6·25, thiamin 1·0, riboflavin 1·625, pyridoxine 1·625, cyanocobalamin 0·015, ascorbic acid 15·0, cholecalciferol 0·3, DL-α-tocopheryl acetate 4·0, biotin 2·5, menadione sodium bisulphite 1·0, nicotinic acid 7·875, pantothenic acid 8·0, pteroylmonoglutamic acid 0·5, *p*-aminobenzoic acid 10·0, inositol 97·5, maize starch 842·81.

gas flow rate was 30 ml N_2/min. The injector temperature was 250° and the detector temperature was 260°. Samples were augmented by 1·0 ml 5α-cholestane (2·5 mg/ml), as internal standard before analysis.

Faeces

Faeces were collected from all groups of pigs for a continuous period of 5 d in week 2 (period 1) and week 6 (period 2). The daily output was weighed and stored at −18°. At the end of the collection periods, faeces from each pig were mixed and a representative sample was taken, freeze-dried for 48 to 72 h and milled for the analyses of faecal steroids and non-starch polysaccharides (NSP).

Ileal digesta

During week 2 (period 1) and week 6 (period 2) the IR pigs were fed at 08.00 and 20.00 hours. For a continuous period of 5 d during these weeks, all digesta produced within this 12 h interval were collected immediately after they appeared and stored at −18°. TiO_2 was added to the diet (1 g/kg) during the whole of the 7 d of weeks 2 and 6 and the total ileal output was calculated on the basis of assumed equal intakes and outputs of the marker. This was carried out to evaluate the closeness to completion of collection of digesta. Total daily output was freeze-dried for 48 to 72 h and milled. Then a single pooled 5-d sample was taken and kept for analysis of steroids and NSP.

Steroids

The analysis of faecal steroids was carried out by the method of Almé *et al.* (1977), as modified by Owen *et al.* (1984). The method consisted of lipid extraction of 500 mg dried samples with 60 ml ethanol (720 ml/l) in a standard Soxhlet apparatus for 12 h. The extracts were filtered and then fractionated by anion-exchange column chromatography on diethylaminohydroxypropyl-Sephadex (DEAP-LH-20; Packard Instruments Ltd, Caver-

sham, Berks). The extract was eluted with a gravity flow of 12–15 ml/h to obtain the total neutral steroids (TNS) fraction. The free bile acid (FBA) fraction was recovered from the column by elution with acetic acid (50 ml, 0·1 M, pH 3·8, in ethanol (720 ml/l)). Fractions were placed in a heating block until completely dry. The TNS fraction was resuspended in 4·0 ml methanol and 5·0 ml diethyl ether, augmented by 1·0 ml methanoic 5α-cholestane (2·5 mg/ml) as internal standard, and quantitatively analysed by GLC as described above for biliary cholesterol. The FBA fraction was resuspended in 1·0 ml methanol, augmented by 1·0 ml methanoic methyl nordeoxycholic acid (1 mg/ml) as internal standard, and methylated with diazomethane before analysis by either GLC or capillary GC. GLC was carried out under the same conditions as described above for biliary cholesterol. For the capillary GC, methyl bile acids were silylated with 1 ml pyridine–hexamethyldisilazane–chlorotrimethylsilane (3:2:1, by vol.) overnight at room temperature; they were then dried and resuspended in hexane. The analysis was carried out on a Carlo Erba HPGC fitted with a capillary column (25 m × 0·2 mm i.d.; 0·25 μ BP1 coating; Scientific Glass Engineering, Sydney, Australia). The temperature gradient was from 200 to 260° at 4°/min, holding at 260° for 45 min. The carrier gas flow rate was 2 ml He/min. The injection temperature was 250° and the detector temperature was 270°. The TNS and FBA fractions of each sample were compared with standards of neutral and acidic steroids respectively, by TLC, before analysis by GLC and capillary GC. The solvent system consisted of ethyl acetate–trimethylpentane–glacial acetic acid (9:9:2 by vol.). The steroids were located using anisaldehyde reagent (2 ml H_2SO_4 + 97 ml acetic acid + 1 ml anisaldehyde) by developing the plates in an oven at 100° for 5–10 min.

NSP

Total and insoluble NSP were analysed by a modified method of Englyst & Cummings (1988). The technique consisted of dispersion and hydrolysis of starch, acidic hydrolysis of NSP, preparation of alditol acetate derivatives, determination of NSP by GLC, and colorimetric determination of uronic acids.

Statistical analysis

Results were analysed by one-way analysis of variance (ANOVA) using the Genstat package (Rothamsted Experimental Station, Harpenden, Herts). When appropriate, the results obtained on day 14 (period 1) were taken as covariate for day 28 and day 42 (period 2). Where the covariate was statistically significant ($P < 0·05$), the adjusted means were used. Mean values of treatments were compared by Student's t test, using appropriate SED values.

RESULTS AND DISCUSSION

Animals

The pigs adapted well to the experimental conditions. Some of them, however, refused part of their diets for the first 2 d of the trial. For these pigs, commercial diet was mixed with experimental diet during those days and was included in the record of feed intake. The IR pigs showed a prompt recovery from surgery and, after 7 d post-operative care, were ready for the trial. Their faecal output was watery throughout the experiment due to the total exclusion of the large intestine, which reduced water absorption. However, as the experiment progressed, adaptation may have occurred as the digesta became thicker. The excretion of digesta caused perianal inflammation and, to relieve the discomfort, these animals were washed and treated in the anal region with healing cream twice daily.

Pigs fed on baked beans (NP and IR) showed lower body weight than BL pigs at the end of the trial (Table 2). Although not to a significant level, the food efficiency ratio (FER) of

Table 2. *Body weight (kg) and food efficiency ratio (FER) of pigs fed on a control diet (BL) and of normal pigs (NP) and pigs with an ileo-rectal anastomosis (IR) fed on a diet containing baked beans* (Phaseolus vulgaris; *300 g/kg*)*

(Mean values for four pigs)

	Body wt (kg)				FER
Day...	1	14	28	42	42
BL	36·0	37·2	46·9†	58·7†[b]	43·4
NP	35·7	37·9	46·6†	55·5†[a]	38·3
IR	35·5	38·7	47·2†	55·6†[a]	42·0
SED (9 df)	2·157	2·156	0·390	0·438	2·021

[a, b] Mean values with different superscript letters in the same column were significantly different (Student's *t* test): $P < 0{\cdot}05$.

FER = body weight gain (kg)/food intake (kg) × 100.

* For details of diets and procedures, see Table 1 and pp. 872–873.

† Mean adjusted for covariate (day 14).

NP pigs was also reduced compared with the other groups. A similar effect of a bean diet on body weight was observed in a previous experiment with pigs (Costa *et al.* 1993) and has also been reported in studies carried out with rats (Chang *et al.* 1986) and humans (Anderson *et al.* 1990). This may be accounted for by the poorer protein quality of the beans compared with casein (the source of protein in the control diet), and possibly to a lower digestibility and/or antinutritional factors present in the beans, although the latter would be low due to the canning process.

Plasma total cholesterol

The results obtained for plasma total cholesterol at fortnightly intervals are given in Table 3. Feeding the control diet for 14 d raised plasma cholesterol of the animals about twofold. However, there was a high variability in responsiveness to dietary cholesterol, as shown by the high values for SED. The existence of hypo- and hyper-responder pigs has been reported by Shutler *et al.* (1988) and Costa *et al.* (1993). Based on those studies, it was anticipated that at least four hyper-responders out of the group of six would be identified after 14 d on the control diet. In the present work, however, fewer pigs developed hypercholesterolaemia. Practical aspects, such as the availability of metabolism cages, time and personnel, allied to the high experimental costs, precluded extra animals being introduced into the experiment to substitute for the hypo-responders in order to reduce the variability. Thus, hypo- and hyper-responders were assigned to experimental groups in such a way that the mean values of plasma total cholesterol between groups were as close as possible.

Consumption of baked beans from day 14 to day 42 reduced plasma cholesterol of NP pigs by 12%. However, no statistical difference was observed between groups, due to the variable response of individuals within groups of small size. Working with a larger number of animals, Shutler *et al.* (1988) and Costa *et al.* (1993) found that baked beans significantly reduced plasma cholesterol when fed to hypercholesterolaemic pigs at a level of 300 g/kg on a dry-matter basis. For pigs with intestinal bypass (IR), baked-bean supplementation exerted no cholesterol-lowering effect, and their plasma cholesterol remained raised throughout the experiment. This fact suggests that the large intestine plays an essential role in the hypocholesterolaemic effect of baked beans.

Table 3. *Plasma metabolite concentrations in pigs fed on a control diet (BL), and in normal pigs (NP) and pigs with an ileo-rectal anastomosis (IR) fed on a diet containing baked beans* (Phaseolus vulgaris; *300 g/kg*)*

(Mean values for four pigs)

Plasma metabolite	Day	Group BL	NP	IR	SED (9 df)
Total cholesterol (mmol/l)	1	2·67	2·58	2·44	0·255
	14	5·02	4·50	4·90	0·954
	28	6·31	3·79	5·76	1·698
	42	6·24†	3·97†	5·58†	1·075
HDL-cholesterol (mmol/l)	1	1·01	1·06	1·03	0·113
	14	1·22	1·44	1·23	0·222
	28	1·71[b]	1·10[a]	1·07[a]	0·263
	42	1·54[b]	1·13[a]	1·19[a]	0·153
HDL-cholesterol: total cholesterol ratio	1	0·38	0·41	0·42	0·040
	14	0·24	0·33	0·26	0·047
	28	0·29	0·30	0·22	0·063
	42	0·24	0·32	0·27	0·065
Triacylglycerol (mmol/l)	1	0·31	0·35	0·23	0·049
	14	0·26	0·26	0·22	0·065
	28	0·30	0·34	0·21	0·061
	42	0·53[b]	0·25[a]	0·28[a]	0·070
Glucose (mmol/l)	1	5·81	5·31	5·06	0·490
	14	6·60	5·42	5·32	0·663
	28	7·58	7·19	6·31	1·295
	42	6·18	5·68	6·88	0·808

HDL, high-density lipoprotein.

[a, b] Means with different superscript letters in the same row were significantly different (Student's *t* test): $P < 0·05$.

* For details of diets and procedures, see Table 1 and pp. 872–875.

† Mean adjusted for covariate (day 14).

HDL-cholesterol

The BL group, fed on the control diet, showed significantly ($P < 0·05$) higher levels of HDL-cholesterol than the other groups on days 28 and 42 (Table 3). This is consistent with a rise in plasma total cholesterol levels. When the HDL-cholesterol: total cholesterol ratio was considered, no significant difference was observed between treatments, although the ratios obtained for pigs fed on the baked-bean diet, particularly NP, were slightly higher than the control. Similar results have been reported for baked beans given to pigs (Shutler *et al.* 1988) and humans (Shutler *et al.* 1989), which suggests a potential of baked beans to reduce the levels of lipoproteins of lower densities (very-low-density-lipoproteins (VLDL) and/or low-density-lipoproteins (LDL)). Indeed, LDL-cholesterol was found to be reduced in pigs given 300 g baked beans/kg, on a dry-matter basis, for 28 d (Costa *et al.* 1993).

Triacylglycerol

Pigs fed on the control diet (BL group) showed an increased plasma triacylglycerol level on day 42. At that stage, triacylglycerol levels of NP and IR pigs were significantly ($P < 0·05$) lower than the control group. This effect does not seem to be related to the bean intake, since the levels of triacylglycerol of pigs NP and IR were not reduced from day 14 to day 42.

Table 4. *Steroid profile of bile (mg/ml) from pigs fed on a control diet (BL) and from normal pigs (NP) and pigs with an ileo-rectal anastomosis (IR) fed on a diet containing baked beans* (Phaseolus vulgaris; *300 g/kg*)*

(Mean values for four pigs)

Steroid	Group			SED (9 df)
	BL	NP	IR	
Cholesterol	0·78	1·58	0·99	0·388
THCA	0·18[a]	0·67[b]	0·43[ab]	0·144
GHCA	1·95[a]	3·32[b]	2·49[ab]	0·580
GCDCA	3·04	3·05	3·42	0·848
PBA	5·17	7·04	6·35	1·420
GHDCA	3·80[b]	7·58[c]	0·34[a]	0·598
TBA	8·97[a]	14·63[b]	6·69[a]	1·884

THCA, taurohyocholic acid; GHCA, glycohyocholic acid; GCDCA, glycochenodeoxycholic acid; PBA, total primary bile acids; GHDCA, glycohyodeoxycholic acid; TBA, total bile acids.

[a, b, c] Means with different superscript letters in the same row were significantly different (Student's *t* test): $P < 0·05$.

* For details of diets and procedures, see Table 1 and pp. 872–875.

Although beans have been reported to lower triacylglycerol levels in hypercholesterolaemic subjects (Anderson *et al.* 1990), the initial levels of triacylglycerol of the pigs in our study were not sufficiently raised to achieve a similar effect.

Biliary steroid profile

The composition of steroids in bile is given in Table 4. The concentration of cholesterol in bile was not found to be significantly different between groups, although the concentration was much higher in the bile of intact pigs fed on baked beans (NP). The effect of beans in increasing the concentration of cholesterol in bile has been demonstrated in humans (Nervi *et al.* 1989) and has been associated with an increased risk of developing gallstones. In rats, newly synthesized cholesterol was found to be preferentially channelled into bile when the animals were fed on a bean diet (Rigotti *et al.* 1989). This may be one of the possible reasons why baked beans reduce plasma cholesterol. In our study, a high variability was found in the concentration of cholesterol in bile of BL and IR pigs. This is reflected in the high values for SED (Table 4) and may have accounted for the non-significance between treatments. It may also be related to the variability in responsiveness to dietary cholesterol.

In terms of bile acids, NP pigs showed a significantly ($P < 0·05$) higher concentration of total bile acids (TBA) in bile than the other groups. The amidated secondary bile acid, glycohyodeoxycholic acid (GHDCA), was the bile acid which contributed most to this increase. Similarly, Hillman *et al.* (1986) found that the highly fermentable soluble fibre, pectin, increased the concentration of the amidated secondary bile acid deoxycholic acid (DCA) in human bile, when compared with the insoluble fibre wheat bran. On the other hand, Pomare & Heaton (1973) reported that insoluble fibre (wheat bran) increased the concentration of chenodeoxycholic acid (CDCA) and reduced the concentration of DCA in human bile. So, the soluble fibre of baked beans may have stimulated absorption of secondary bile acids in the large bowel and increased their concentration in bile. This effect seems to be similar for humans and pigs, apart from the fact that pigs produce hyodeoxycholic acid (HDCA) and not DCA as the major secondary bile acid.

Table 5. *Daily output (g) on a dry-matter basis, of faeces and neutral steroids, by pigs fed on a control diet (BL) and by normal pigs (NP) and pigs with an ileo-rectal anastomosis (IR) fed on a diet containing baked beans* (Phaseolus vulgaris; *300 g/kg*), *during two periods of faecal collection**

(Mean values for four pigs)

Group	Faeces	Cholesterol	Coprostanol	Total animal steroids	Plant steroids	Total neutral steroids
Period 1						
BL	224·2	3·78	1·36[b]	5·14	0·54	5·68
NP	199·9	3·78	1·44[b]	5·22	0·59	5·81
IR	246·6	5·93	0·31[a]	6·24	0·52	6·76
SED (9 df)	22·72	0·984	0·284	1·123	0·172	1·259
Period 2						
BL	283·2†[ab]	6·70[b]	2·18[b]	8·88	1·46	10·34
NP	234·4†[a]	3·72[a]	4·06[c]	7·78	1·34	9·12
IR	315·8†[b]	9·16[c]	0·32[a]	9·48	1·28	10·77
SED (9 df)	15·23	0·892	0·360	0·988	0·477	1·295

[a, b, c] Means with different superscript letters in the same column were significantly different (Student's *t* test): $P < 0·05$.

* For details of diets and procedures, see Table 1 and pp. 872–875.

† Means adjusted for covariate (Period 1).

The major primary bile acids synthesized in the pig liver are hyocholic acid (HCA) and CDCA. The concentration of total primary bile acid (PBA) was not significantly affected by the bean consumption. Nevertheless, some differences were observed when primary bile acids were analysed individually. For instance, intact pigs on the baked-bean diet (NP) showed higher levels ($P < 0·05$) of taurohyocholic acid (THCA) and glycohyocholic acid (GHCA) than pigs on the control diet (BL), but the glycochenodeoxycholic acid (GCDCA) concentration did not differ between them. No statistical difference was observed between IR and NP pigs, both fed on baked beans, in terms of THCA, GHCA and GCDCA. This suggests that the total exclusion of the large intestine did not affect the absorption and/or secretion of primary bile acids. The effect of baked beans in the diet seems to be associated with the bile acids that escape absorption in the small intestine and undergo transformation by the bacterial flora in the large intestine.

Faecal output

The daily outputs of faeces during periods 1 and 2 of collection are shown in Table 5. No statistical difference was observed between groups in period 1. In period 2, however, IR pigs fed on baked beans excreted significantly ($P < 0·05$) more faeces than the intact pigs (NP) fed on the same diet. Therefore, some components of baked beans not digested in the small intestine were degraded by colonic flora in the large intestine. The outputs for NP and BL pigs were not significantly different in both periods, which suggests similar bulking capacity of the beans to cellulose (Solka-floc). A low bulking capacity of beans has been observed in a study with humans (Anderson *et al.* 1984).

Faecal steroids

Daily output of neutral steroids of pigs in periods 1 and 2 of collection is shown in Table 5. No difference between treatments was obtained for total neutral steroids, plant steroids and total animal steroids in either period of analysis. Similarly, Anderson *et al.* (1984)

Table 6. *Daily output (g) on a dry-matter basis, of acidic steroids, by pigs fed on a control diet (BL) and by normal pigs (NP) and pigs with an ileo-rectal anastomosis (IR) fed on a diet containing baked beans* (Phaseolus vulgaris; *300 g/kg*), *during two periods of faecal collection**

(Mean values for four pigs)

Group	CDCA	HCA	PBA	LCA	HDCA	UDCA	OBA	SBA	TBA
Period 1									
BL	0·00[a]	0·00[a]	0·00[a]	0·29[b]	0·53[b]	0·00	0·03	0·85[b]	0·85
NP	0·00[a]	0·00[a]	0·00[a]	0·29[b]	0·57[b]	0·00	0·04	0·90[b]	0·90
IR	0·08[b]	0·73[b]	0·81[b]	0·04[a]	0·13[a]	0·03	0·04	0·24[a]	1·05
SED (9 df)	0·020	0·241	0·261	0·052	0·120	0·012	0·030	0·179	0·257
Period 2									
BL	0·00[a]	0·00[a]	0·00[a]	0·40[c]	0·65[b]	0·00	0·03[a]	1·08[b]	1·08[b]
NP	0·00[a]	0·00[a]	0·00[a]	0·18[b]	0·24[a]	0·00	0·02[a]	0·44[a]	0·44[a]
IR	0·21[b]	1·54[b]	1·75[b]	0·05[a]	0·13[a]	0·05	0·10[b]	0·33[a]	2·08[c]
SED (9 df)	0·036	0·156	0·183	0·048	0·106	0·023	0·020	0·166	0·238

CDCA, chenodeoxycholic acid; HCA, hyocholic acid; PBA, total primary bile acids; LCA, lithocholic acid; HDCA, hyodeoxycholic acid; UDCA, ursodeoxycholic acid; OBA, other bile acids; SBA, total secondary bile acids; TBA, total bile acids.

[a, b, c] Means with different superscript letters in the same column were significantly different (Student's *t* test): $P < 0.05$.

* For details of diets and procedures, see Table 1 and pp. 872–875.

found no effect on the excretion of neutral steroids in hypercholesterolaemic subjects receiving bean or oat diets. When the animal steroids (coprostanol and cholesterol) were analysed individually, some remarkable differences were observed. For instance, the normal pigs fed on baked beans (NP) presented a significantly higher ($P < 0.05$) excretion of coprostanol when compared with both BL and IR pigs, in period 2. They also excreted significantly lower levels of cholesterol in the same period. This fact implies that baked beans potentiate the conversion of cholesterol to coprostanol, probably by increasing bacterial mass. Vahouny *et al.* (1987) also found an increased conversion of cholesterol to coprostanol in rats fed on a pectin-containing diet when compared with one containing cellulose. Thus, the fermentation of soluble fibre seems to affect the metabolism of steroids in the large bowel. The anastomosed pigs (IR), on the other hand, excreted significantly lower amounts of coprostanol in both periods of faecal collection. This result was expected, since the conversion of cholesterol to coprostanol occurs in the large intestine. Their cholesterol output was significantly higher ($P < 0.05$) than the other groups, in period 2, due to impaired conversion to coprostanol by the flora in the large intestine.

The daily output of acidic steroids is given in Table 6. The output of TBA was not statistically different in period 1 when pigs were fed on the same control diet. Nevertheless, the intake of baked beans (period 2) caused some changes in this profile. For instance, the excretion of TBA and PBA of IR pigs was higher. NP pigs, on the other hand, eliminated significantly lower ($P < 0.05$) levels of TBA, especially secondary bile acids (SBA), than the control pigs. This finding suggests that absorption and recycling of bile acids may be increased in normal pigs fed on baked beans, because the SBA concentration in the bile was also increased in these pigs (Table 4). Previous reports on the effect of soluble fibre on bile-acid metabolism are contradictory and therefore not conclusive. Pectin was found either to increase (Reddy *et al.* 1980), to reduce (Vahouny *et al.* 1987), or not to affect (Sharma, 1984) bile-acid excretion in rats when compared with cellulose. Sharma (1985) also did

Table 7. *Daily output (g) of starch residue (glucose) by pigs fed on a control diet (BL) and by normal pigs (NP) and pigs with an ileo-rectal anastomosis (IR) fed on a diet containing baked beans* (Phaseolus vulgaris; *300 g/kg*), *during two periods of faecal collection**

(Mean values for four pigs)

Period	BL	NP	IR	SED (9 df)
1	1·53[a]	1·70[a]	6·13[b]	1·516
2	1·09[a]	0·08[a]	25·63[b]	2·382

[a, b] Means with different superscript letters in the same row were significantly different (Student's *t* test): $P < 0{\cdot}05$.

* For details of diets and procedures, see Table 1 and pp. 872–875.

not find an increased excretion of bile acids when the soluble fibre gum acacia was fed to humans. Thus, these results reinforce those of Vahouny *et al.* (1987) and Story (1986) that fibre-induced changes in faecal bile acid content, concentration or composition may not be the sole mechanism involved in lowering plasma cholesterol levels. The cholesterol-lowering effect of baked beans may be due not to an increased output of steroids but to an influence on their enterohepatic circulation mediated via large bowel absorption.

Starch and NSP

The output of starch residue in periods 1 and 2 of faecal collection is shown in Table 7. The daily output of starch of the pigs with intestinal anastomosis (IR) was greater than the other pigs, in both periods. However, in period 2 the excretion was much higher, due to the baked bean substitution. This implies that much of the starch in a baked-bean diet resists digestion in the small intestine and becomes available for fermentation in the large intestine. The reason for incomplete digestion of starch in the small intestine is not clear. Englyst & Kingman (1990) reported that it may be due to intrinsic (physical inaccessibility, granular structure of the starch, retrogradation, α-amylase inhibitors) and extrinsic (extent of chewing, transit time, availability of α-amylase) factors. The resistant starch may be fermented in the large bowel, with production of volatile fatty acids (VFA; Andrieux *et al.* 1992). In our study it was found that 99·7% of the starch which escaped small intestinal digestion was fermented in the large intestine. This was shown by the different outputs of NP and IR pigs in period 2.

The output of total NSP residues in periods 1 and 2 of faecal collection is given in Table 8. No statistical difference was observed between groups in period 1, except for rhamnose. The intestinal anastomosis of pigs IR did not affect daily output of NSP, which suggests low fermentation of cellulose (Solka-floc) in the hind-gut. In period 2, NP pigs showed significantly ($P < 0{\cdot}05$) lower excretion of total residues of NSP than the BL group. Therefore, soluble fibre from baked beans was degraded to a greater extent than insoluble fibre present in the control diet. Similarly, an isolated soluble fibre, pectin, was found to stimulate microbial fermentation in pigs (Fleming & Wasilewski, 1984) and in humans (Marthinsen & Fleming, 1982). In the present experiment it was found that 81·9% of the baked-bean NSP which reached the large bowel was fermented. This is shown by the comparison between NP and IR pigs in period 2. IR pigs showed lower output of total NSP than BL pigs, which suggests partial degradation of soluble NSP in the small intestine of those animals. The higher outputs of rhamnose, fucose, galactose and uronic acids for IR pigs, compared with BL pigs, is due to the composition of NSP of baked beans compared with Solka-floc.

Table 8. *Daily output (g) of total non-starch polysaccharide (NSP) residues by pigs fed on a control diet (BL) and by normal pigs (NP) and pigs with an ileo-rectal anastomosis (IR) fed on a diet containing baked beans* (Phaseolus vulgaris; *300 g/kg*), *during two periods of faecal collection**

(Mean values for four pigs)

Group	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acids	Total NSP
Period 1									
BL	0·83[b]	0·00	0·20	3·42	0·86	1·11	40·46	2·42	49·44
NP	0·29[a]	0·00	0·21	2·75	0·75	0·93	34·33	2·04	41·31
IR	0·78[b]	0·00	0·25	5·03	0·96	1·13	46·07	1·95	56·17
SED (9 df)	0·157	0·000	0·039	0·839	0·169	0·118	8·900	0·222	10·160
Period 2									
BL	1·11[a]	0·00[a]	0·26	3·20†[b]	1·07†[c]	1·27†[a]	48·01[c]	2·20[a]	57·13[c]
NP	0·90[a]	0·14[a]	0·21	0·69†[a]	0·32†[a]	1·59†[a]	1·74[a]	1·76[a]	6·70[a]
IR	1·76[b]	0·55[b]	2·51	4·00†[b]	0·76†[b]	2·37†[b]	18·82[b]	5·64[b]	37·06[b]
SED (9 df)	0·149	0·113	1·246	0·433	0·085	0·270	3·150	0·776	4·900

[a, b, c] Means with different superscript letters in the same column were significantly different (Student's *t* test): $P < 0·05$.

* For details of diets and procedures, see Table 1 and pp. 872–875.

† Mean adjusted for covariate (Period 1).

Table 9. *Daily output (g) of soluble non-starch polysaccharide (NSP) residues by pigs fed on a control diet (BL) and by normal pigs (NP) and pigs with an ileo-rectal anastomosis (IR) fed on a diet containing baked beans* (Phaseolus vulgaris; *300 g/kg*), *during two periods of faecal collection**

(Mean values for four pigs)

Group	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acids	Total NSP
Period 1									
BL	0·75[b]	0·00	0·00	0·36	0·11	1·05	0·61	2·42	5·45
NP	0·15[a]	0·00	0·08	0·33	0·14	0·85	0·65	2·04	4·25
IR	0·78[b]	0·00	0·11	0·44	0·07	1·09	0·00	1·95	4·44
SED (9 df)	0·176	0·000	0·065	0·067	0·034	0·107	0·366	0·222	0·662
Period 2									
BL	1·00†[a]	0·00[a]	0·00	0·33	0·14[a]	1·21†	0·60	2·20[a]	5·70[a]
NP	1·07†[a]	0·14[a]	0·00	0·18	0·28[ab]	1·41†	0·80	1·76[a]	5·12[a]
IR	1·63†[b]	0·47[b]	2·09	0·82	0·39[b]	1·74†	1·18	5·64[b]	14·27[b]
SED (9 df)	0·190	0·088	1·026	0·251	0·072	0·231	0·524	0·776	2·600

[a, b, c] Means with different superscript letters in the same column were significantly different (Student's *t* test): $P < 0·05$.

* For details of diets and procedures, see Table 1 and pp. 872–875.

† Mean adjusted for covariate (Period 1).

In terms of soluble NSP (Table 9), apart from rhamnose, no significant difference was observed between groups in period 1. In period 2, the outputs of rhamnose, fucose and uronic acids were significantly higher in IR than NP pigs. This suggests that such residues underwent greater fermentation in the large bowel of intact pigs, receiving baked beans.

Table 10. *Daily intake (g) of food, Solka-floc (cellulose), baked beans* (Phaseolus vulgaris) *and non-starch polysaccharide (NSP) residues, on a dry-matter basis, of pigs fed on a control diet (BL) and of normal pigs (NP) and pigs with an ileo-rectal anastomosis (IR) fed on a diet containing baked beans (300 g/kg)**

(Mean values for four pigs)

Pig	Food	Solka-floc	Baked beans	Rha	Fuc	Ara	Xyl	Man	Gal	Glu	UAc	Total NSP	% AD
Period 1													
BL	1030	58·7	—	0·00	0·00	0·27	10·25	0·60	0·15	46·1	ND	57·4	16·8
NP	1048	59·7	—	0·00	0·00	0·27	10·43	0·61	0·15	46·9	ND	58·4	33·2
IR	1071	61·0	—	0·00	0·00	0·28	10·65	0·62	0·16	47·9	ND	59·6	10·3
SED (9 df)	59·9	ND	ND	0·000	0·000	0·016	0·595	0·035	0·009	2·67	ND	3·33	15·75
Period 2													
BL	1419	80·9	—	0·00[a]	0·00[a]	0·55†[a]	14·13†[b]	0·84†[a]	0·25†[a]	64·17†[b]	ND	80·4†[b]	30·2[a]
NP	1395	—	452·7	0·90[b]	1·36[b]	21·78†[b]	6·80†[a]	0·91†[b]	3·62†[b]	15·44†[a]	9·96[a]	60·8†[a]	88·9[b]
IR	1412	—	458·5	0·91[b]	1·37[b]	21·78†[b]	6·66†[a]	0·90†[b]	3·63†[b]	14·86†[a]	10·08[a]	60·0†[a]	39·9[a]
SED (9 df)	90·4	ND	ND	0·048	0·073	0·838	0·203	0·018	0·145	1·318	0·533	1·044	6·22

Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose, UAc, uronic acids; AD, apparent digestibility of total NSP; ND, not determined.

[a, b] Means with different superscript letters in the same column were significantly different (Student's *t* test): $P < 0{\cdot}05$.

* For details of diets and procedures, see Table 1 and pp. 872–875.

† Mean adjusted for covariate (Period 1).

From the soluble NSP which passed to the large intestine in the pigs fed on baked beans, 64·1% was degraded. The fermentation of fibre in the pig intestine is known to produce VFA, which can be absorbed and contribute to available energy (Ehle *et al.* 1982; Vervaeke *et al.* 1989). In our study, however, the intact pigs fed on baked beans did not show a significant difference in body weight, compared with the other groups. VFA, particularly propionic acid, have been reported to inhibit cholesterol synthesis and, therefore, reduce plasma cholesterol in hypercholesterolaemic pigs (Boila *et al.* 1981; Thacker *et al.* 1981). Although not determined in the present investigation, the production of VFA by NP pigs fed on baked beans may exert a plasma-cholesterol-lowering effect.

The intake of NSP residues and the apparent digestibility of total NSP is shown in Table 10. NP pigs fed on baked beans (period 2) consumed more rhamnose, fucose, arabinose, mannose, galactose, and uronic acids than pigs consuming Solka-floc (BL) and showed higher apparent digestibility of NSP. Digestibility of NSP was also reported by Goodlad & Mathers (1991) to be higher in pigs fed on legumes (peas) than insoluble fibre (wheat bran). BL pigs presented lower apparent digestibility, especially for glucose and xylose. These monomers were also shown by Longland & Low (1988) to be poorly digestible. Xylose is presumably bound to cellulose and not accessible for microbial degradation. Although the consumption of arabinose by pigs on the baked-bean diet was about 22 g/d, the output (period 2) was very low, even for the IR pigs. This suggests that arabinose could have been mostly degraded in the small intestine. The outputs of rhamnose, mannose and galactose of pigs on Solka-floc were higher than their intakes for the corresponding period of analysis. Such monomers were found from microbial cultures in pig faeces (Longland & Low, 1988). So, the faecal microbes and possibly intestinal cell sloughing may have contributed to the increased excretion of these NSP residues.

CONCLUSION

Story & Kritchevsky (1978) pointed out that 'changes in bile salt metabolism that result from changes in the type and amount of fibre administered seem to be related to the levels of cholesterol in blood and bile'. In fact, in the present study the change in the type of dietary fibre was followed by remarkable changes in bile acid metabolism which, in turn, was reflected in different levels of cholesterol in blood and bile. Although the plasma cholesterol reduction of pigs fed with 300 g baked beans/kg diet was not found here to be significant, this effect is supported by our previous study (Costa *et al.* 1993) and others (Shutler *et al.* 1988, 1989).

Some results obtained in the present investigation relevant to the effects of baked-bean consumption by intact pigs (NP) are: (*a*) reduced plasma cholesterol (NS); (*b*) increased concentration of cholesterol (NS) and total bile acids, especially the secondary GHDCA in bile ($P < 0·05$); (*c*) increased conversion of cholesterol to coprostanol in the large intestine ($P < 0·05$); (*d*) reduced bile acid excretion, especially secondary bile acids in faeces ($P < 0·05$); and (*e*) reduced output and increased apparent digestibility of NSP ($P < 0·05$). These findings suggest that baked beans potentiate bacterial fermentation and steroid degradation in the large intestine. Simultaneously, they seem to enhance conservation of bile acids and cholesterol in the enterohepatic circulation. The high concentration of bile acids and cholesterol in bile of animals fed on baked beans may promote a feedback inhibition of cholesterol synthesis in the liver and, hence, reduce plasma cholesterol. Although bile acids are reported to suppress cholesterol synthesis in liver (Grundy, 1978), other mechanisms may also be involved synergistically in the cholesterol-lowering effect of baked beans. Some legume constituents not analysed here, such as protein (Kritchevsky, 1979; Kim *et al.* 1980), saponins (Oakenfull *et al.* 1979) and the fibre fermentation product

propionic acid (Chen *et al.* 1984; Illman *et al.* 1988), have been considered to be hypocholesterolaemic agents. Thus, to elucidate the mechanisms by which baked beans reduce plasma cholesterol, further investigations are necessary.

This work was supported by CNPq, Brazil and the International Foundation for the Promotion of Nutrition Research and Nutrition Education, Switzerland. The authors wish to thank the Agricultural and Food Research Council for providing the facilities for pig experimentation, Mr H. Keal for the blood sampling, Dr H. Buttle for the animal surgery, Dr S. Abeyasekera for the statistical advice and Mrs J. Carr for valuable technical assistance.

REFERENCES

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. & Fu, P. C. (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry* **20**, 470–475.

Almé, B. A., Bremmelgaard, A., Sjovall, J. & Thomassen, P. (1977). Analysis of metabolic profiles of bile acids in urine using lipophilic anion exchange and computerised gas–liquid chromatography–mass spectrometry. *Journal of Lipid Research* **18**, 339–362.

Anderson, J. W. & Chen, W. J. L. (1979). Plant fiber. Carbohydrate and lipid metabolism. *American Journal of Clinical Nutrition* **32**, 346–363.

Anderson, J. W., Gustafson, N. J., Spencer, D. B., Tietyen, J. & Bryant, C. A. (1990). Serum lipid response of hypercholesterolemic men to single and divided doses of canned beans. *American Journal of Clinical Nutrition* **51**, 1013–1019.

Anderson, J. W., Story, L., Sieling, B., Chen, W. J. L., Petro, M. S. & Story, J. A. (1984). Hypocholesterolemic effects of oat bran or bean intake for hypercholesterolemic men. *American Journal of Clinical Nutrition* **40**, 1146–1155.

Andrieux, C., Pacheco, E. D., Bouchet, B., Gallant, D. & Szylit, O. (1992). Contribution of the digestive tract microflora to amylomaize starch degradation in the rat. *British Journal of Nutrition* **67**, 489–499.

Boila, R. J., Salomons, M. O., Milligan, L. P. & Aherne, F. X. (1981). The effect of dietary propionic acid on cholesterol synthesis in swine. *Nutrition Reports International* **23**, 1113–1121.

Chang, K. C., Ethen, S., Harrold, R. & Brown, G. (1986). Effect of feeding dry beans on rat plasma cholesterol. *Nutrition Reports International* **33**, 659–664.

Chen, W. L., Anderson, J. W. & Jennings, D. (1984). Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol-fed rats. *Proceedings of the Society for Experimental Biology and Medicine* **175**, 215–218.

Costa, N. M. B., Walker, A. F. & Low, A. G. (1993). The effect of graded inclusion of baked beans on plasma and liver lipids in hypercholesterolaemic pigs given a Western-type diet. *British Journal of Nutrition* **70**, 515–524.

Ehle, F. R., Jeraci, J. L., Robertson, J. B. & Van Soest, P. J. (1982). The influence of dietary fibre on digestibility, rate of passage and gastrointestinal fermentation in pig. *Journal of Animal Science* **55**, 1071–1081.

Englyst, H. N. & Cummings, J. H. (1988). Improved method for measurement of dietary fiber as non-starch polysaccharides in plant food. *Journal of the Association of Official Analytical Chemists* **71**, 808–814.

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

Fleming, S. E. & Wasilewski, M. M. (1984). Using the pig as a tool for studying fermentation in the human gut. *Nutrition Reports International* **30**, 825–834.

Fossati, P. & Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry* **28**, 2077–2080.

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

Green, S., Bertrand, S. L., Duron, M. J. C. & Maillard, R. A. (1987). Digestibility of amino acids in maize, wheat and barley meal, measured in pigs with ileo-rectal anastomosis and isolation of the large intestine. *Journal of the Science of Food and Agriculture* **40**, 29–43.

Grundy, S. M. (1978). Cholesterol metabolism in man. *Western Journal of Medicine* **128**, 13–25.

Hillman, L. C., Peters, S. G., Fisher, C. A. & Pomare, E. W. (1986). Effects of the fibre components pectin, cellulose, and lignin on bile salt metabolism and biliary lipid composition in man. *Gut* **27**, 29–36.

Houghton, P. W. J., Owen, R. W., Mortensen, N. J. McC., Hill, M. J. & Williamson, R. C. N. (1989). Gastroduodenal and faecal bile acid metabolism after gastric surgery: implications for colonic and gastric carcinogenesis. *Surgical Research Communications* **6**, 303–311.

Illman, R. J., Topping, D. L., McIntosh, G. H., Trimble, R. P., Storer, G. B., Taylor, M. N. & Cheng, B. Q. (1988). Hypocholesterolaemic effects of dietary propionate: studies in whole animals and perfused rat liver. *Annals of Nutrition and Metabolism* **32**, 97–107.

Kim, D. N., Lee, K. T., Reiner, J. M. & Thomas, W. A. (1980). Increased steroid excretion in swine fed high-fat, high cholesterol diet with soy protein. *Experimental and Molecular Pathology* **33**, 25–35.
Kingman, S. M. (1991). The influence of legume seeds on human plasma lipid concentrations. *Nutrition Research Reviews* **4**, 97–123.
Kritchevsky, D. (1979). Vegetable protein and atherosclerosis. *Journal of the American Oil Chemists Society* **56**, 135–140.
Longland, A. C. & Low, A. G. (1988). Digestion of diets containing molassed or plain sugar-beet pulp by growing pigs. *Animal Feed Science and Technology* **23**, 67–78.
Marthinsen, D. & Fleming, S. E. (1982). Excretion of breath and flatus gases by humans consuming high-fiber diets. *Journal of Nutrition* **112**, 1133–1143.
Nervi, F., Covarrubias, C., Bravo, P., Velasco, N., Ulloa, N., Cruz, F., Fava, M., Severin, C., Del Pozo, R., Antezana, C., Valvidieso, V. P. & Artega, A. (1989). Influence of legume intake on biliary lipids and cholesterol saturation in young Chilean men. *Gastroenterology* **96**, 825–830.
Oakenfull, D. G., Fenwick, D. E., Hood, R. L., Topping, D. L., Illman, R. J. & Storer, G. B. (1979). Effects of saponins on bile acids and plasma lipids in the rat. *British Journal of Nutrition* **42**, 209–216.
Owen, R. W., Thompson, M. H. & Hill, M. J. (1984). Analysis of metabolic profiles of steroids in faeces of healthy subjects undergoing chenodeoxycholic acid treatment by liquid-gel chromatography–mass spectrometry. *Journal of Steroid Biochemistry* **21**, 593–600.
Pomare, E. W. & Heaton, K. W. (1973). Alteration of bile salt metabolism by dietary fiber (bran). *British Medical Journal* **4**, 262–264.
Reddy, B. S., Watanabe, K. & Sheinfil, A. (1980). Effect of dietary wheat bran, alfalfa, pectin and carrageenan on plasma cholesterol and faecal bile acid and neutral sterol excretion in rats. *Journal of Nutrition* **110**, 1247–1254.
Rigotti, A., Marzolo, M. P., Ulloa, N., Gonzalez, O. & Nervi, F. (1989). Effect of bean intake on biliary lipid secretion and on hepatic cholesterol metabolism in the rat. *Journal of Lipid Research* **30**, 1041–1048.
Sharma, R. D. (1984). Hypocholesterolemic effect of hydroxy acid components of Bengal gram. *Nutrition Reports International* **29**, 1315–1322.
Sharma, R. D. (1985). Hypocholesterolemic effect of gum acacia in men. *Nutrition Research* **5**, 1321–1326.
Shutler, S. M., Bircher, G. M., Tredger, J. A., Morgan, L. M., Walker, A. F. & Low, A. G. (1989). The effect of daily baked bean (*Phaseolus vulgaris*) consumption on the plasma lipid levels of young, normo-cholesterolaemic men. *British Journal of Nutrition* **61**, 257–265.
Shutler, S. M., Low, A. G. & Walker, A. F. (1988). Influence of baked beans on plasma lipids in pigs fed on a hypercholesterolaemic diet. *Proceedings of the Nutrition Society* **47**, 97A.
Shutler, S. M., Walker, A. F. & Low, A. G. (1987*a*). The cholesterol-lowering effects of legumes. I. Effects of the major nutrients. *Human Nutrition: Food Sciences and Nutrition* **41F**, 71–86.
Shutler, S. M., Walker, A. F. & Low, A. G. (1987*b*). The cholesterol-lowering effects of legumes. II. Effects of fibre, sterols, saponins and isoflavones. *Human Nutrition: Food Sciences and Nutrition* **41F**, 87–102.
Sidhu, G. S., Upson, B. & Malinow, M. R. (1987). Effects of soy saponins and tigogenin cellobioside on intestinal uptake of cholesterol, cholate and glucose. *Nutrition Reports International* **35**, 615–623.
Spigelman, A. D., Owen, R. W., Hill, M. J. & Phillips, R. K. S. (1991). Biliary bile acid profiles in familial adenomatous polyposis. *British Journal of Surgery* **78**, 321–325.
Story, J. A. (1986). Modification of steroid excretion in response to dietary fiber. In *Dietary Fiber. Basic and Clinical Aspects* [G. V. Vahouny & D. Kritchevsky, editors]. New York: Plenum.
Story, J. A. & Kritchevsky, D. (1978). Bile acid metabolism and fiber. *American Journal of Clinical Nutrition* **31**, S199–S202.
Thacker, P. A., Salomons, M. O., Aherne, F. X., Milligan, L. P. & Bowland, J. P. (1981). Influence of propionic acid on the cholesterol metabolism of pigs fed hypercholesterolemic diets. *Canadian Journal of Animal Science* **61**, 969–975.
Vahouny, G. V., Khalafi, R., Satchithanandan, S., Watkins, D. W., Story, J. A., Cassidy, M. M. & Kritchevsky, D. (1987). Dietary fiber supplementation and fecal bile acids, neutral steroids, and divalent cations in rats. *Journal of Nutrition* **117**, 2009–2015.
Vervaeke, I. J., Dierick, N. A., Demeyer, D. I. & Decuypere, J. A. (1989). Approach to the energetic importance of fibre digestion in pigs. II. An experimental approach to hindgut digestion. *Animal Feed Science and Technology* **23**, 169–194.
Warnick, G. R., Benderson, J. & Albers, J. J. (1982). Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clinical Chemistry* **28**, 1379–1388.

Printed in Great Britain