Comparative effects of four legume species on plasma lipids and faecal steroid excretion in hypercholesterolaemic pigs

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The effect of four species of legume seeds on plasma cholesterol levels and faecal steroid excretion was studied in pigs. Thirty-six growing boars were randomly allocated in groups of six to six diets which they ate continuously for 42 d. The diets fed were: 1, a semi-purified (SP; control group 1) diet; 2, SP + 10 g cholesterol/kg (control group 2); 3, 4, 5, 6, SP + cooked legumes (70:30, w/w; respectively baked beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), lentils (*Lens culinaris* Medik.), butter beans (*Phaseolus lunatus*)) + 10 g cholesterol/kg. Fasting blood samples were taken on days 0, 14, 28, and 42 for the determination of total plasma cholesterol, high-density-lipoprotein (HDL)-cholesterol and triacylglycerols. Between days 7 and 11 and days 28 and 32 complete 5 d faecal collections were made for the measurement of neutral, acidic and conjugated steroids. After 42 d total cholesterol and HDL-cholesterol levels were raised significantly in all groups, but to different extents. In comparison with control group 2, diet-induced hypercholesterolaemia was significantly inhibited in the groups consuming baked beans, peas and butter beans, although HDL-cholesterol levels were maintained. Faecal steroid excretion by the legume groups was not significantly different from that of control group 2. The results suggest that the mechanism for the hypocholesterolaemic effect does not involve increased hepatic bile acid synthesis and thereby increased cholesterol clearance via the intestinal route.

Legumes: Hypocholesterolaemic effect: Lipid metabolism: Pigs

The mature seeds of several legume species have been shown to reduce plasma cholesterol levels in experimental animals and man (for a review, see Shutler *et al.* 1987). The effect is particularly marked in hyperlipidaemic and cholesterol-fed subjects. Some of the studies in rats on this effect have demonstrated a commensurate increase in the excretion of bile acids in the faeces in response to whole legume seeds or fractions of these (Mathur *et al.* 1964; Soni *et al.* 1982; Mahadevappa & Raina, 1983). This increased excretion of steroids has, therefore, been implicated as the mechanism involved, and this has been associated variously with the dietary fibre, saponin or lipid fractions of the seeds.

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Few of the studies on the hypolipidaemic effect of leguminous diets in humans have included measurements of faecal steroid excretion. Mathur *et al.* (1968) observed that substitution of chick peas (*Cicer arietinum* L.; Bengal gram) into a high-butter-fat diet significantly increased 24 h excretion of bile acids but did not affect neutral sterol excretion. However, the diets were not completely standardized in this study for their carbohydrate, protein and fat contents. In contrast, in the carefully controlled study of Anderson *et al.* (1984) using *Phaseolus vulgaris* (haricot beans) the excretion of bile acids in the faeces of human hyperlipidaemic subjects was reduced by 30%, despite a decrease in plasma cholesterol. Similar observations have been made in studies using isolated soya-bean protein (Fumagalli *et al.* 1982).

The rat is not an ideal model for human lipid metabolism, having a dissimilar plasma lipid profile and responding poorly to atherogenic diets. It is, thus, possible that the reported increase in bile acid excretion by rats in response to legumes does not occur in humans. Indeed, the assumption of similarity between the species may have delayed investigation into the hypolipidaemic mechanism occurring in man.

In contrast to the rat, the pig has a plasma lipid profile very similar to that of man and responds quickly to hyperlipidaemic diets (Miller & Ullrey, 1987). The object of the present study was to investigate the effects of the mature seeds from four different legume species on plasma lipid levels and faecal steroid excretion in pigs made hypercholesterolaemic by diet. The legume seeds used were analysed so that any differences in composition of their components might be related to the physiological effects they elicited.

MATERIALS AND METHODS

Animals

Thirty-six growing Large White × Landrace boars of starting weight 25–34 kg were used. These were housed in single floor pens or metabolic crates and maintained at $20 \pm 3°$.

Diets

The formulations of the diets used are given in Table 1. Each diet was designed to provide approximately 12% of energy as protein, 40% of energy as fat (P:S ratio 0·31) and 48% of energy as carbohydrate, in imitation of a typical UK dietary profile (Department of Health and Social Security, 1984). Diets 2–6 were supplemented with 10 g crystalline cholesterol/kg which was mixed thoroughly with the soya-bean oil before preparation of the diets. The diets were fed at a level of 30 g/kg body weight per d (dry weight) in two equal meals, with tap water (2·5 l/kg).

Marrowfat peas (*Pisum sativum* L.), red lentils (*Lens culinaris* Medik.) and butter beans (*Phaseolus lunatus* L.) were obtained dry, soaked overnight in deionized water, boiled until soft, and stored at $-15°$ until required. Baked beans (*Phaseolus vulgaris* L.) in tomato sauce were obtained in catering cans.

Diet treatments and sampling

The animals were randomly allocated to six groups of six. Each group received the same diet for a period of 6 weeks: the animals were weighed weekly for assessment of diet requirements. Groups 1 and 2 received semi-purified diets (diet 1 without and diet 2 with added cholesterol) and acted as controls. Groups 3–6 received cholesterol-supplemented semi-purified diets together with the following cooked legumes (300 g/kg dry matter): baked beans (diet 3), marrowfat peas (diet 4), red lentils (diet 5), butter beans (diet 6).

Table 1. *Formulations of the diets (g/kg dry matter)*

Diet	1	2	3	4	5	6
Maize starch	477·0	477·9	352·0	336·8	347·5	335·0
Sucrose	84·0	84·0	62·0	86·7	86·7	86·7
Tallow	130·0	130·0	133·0	133·0	133·0	133·0
Soya-bean oil	45·0	45·0	42·8	42·8	42·8	42·8
Casein	157·0	157·0	76·0	66·4	55·7	68·2
Cellulose	57·0	57·0	—	—	—	—
Baked beans (*Phaseolus vulgaris*)	—	—	300·0	—	—	—
Marrowfat peas (*Pisum sativum*)	—	—	—	300·0	—	—
Red lentils (*Lens culinaris* Medik.)	—	—	—	—	300·0	—
Butter beans (*Phaseolus lunatus*)	—	—	—	—	—	300·0
Mineral mix	10·0	10·0	07·0	07·0	07·0	07·0
Vitamin mix	02·0	02·0	01·4	01·4	01·4	01·4
Sodium chloride	05·0	05·0	03·5	03·5	03·5	03·5
Choline chloride	01·1	01·1	00·8	00·8	00·8	00·8
Dicalcium phosphate	31·0	31·0	21·7	21·7	21·7	21·7

Fasting blood samples were taken by venepuncture from the anterior vena cava on days 0, 14, 28 and 42 of the study. Complete 5 d faecal collections were made between days 7 and 11 (period 1) and between days 28 and 32 (period 2).

Analyses

Plasma was separated from the whole blood samples by low-speed centrifugation immediately after collection and stored frozen before analysis. Plasma cholesterol, high-density-lipoprotein (HDL)-cholesterol and triacylglycerols were measured using a fully automated clinical analyser (Encore Clinical Chemistry System; Baker Instruments) and enzymic kits, as described previously (Shutler *et al.* 1989). The very-low-density lipoprotein (VLDL) + low-density lipoprotein (LDL) combined fraction was determined by difference.

Inter- and intra-assay coefficients of variation (%) for the analyses were: for cholesterol 1·8 and 1·1 respectively, and for triacylglycerols 3·6 and 2·8 respectively.

A representative sample of each 5 d faecal collection was freeze-dried and milled to a fine powder. Samples were analysed for neutral sterols, free bile acids and glycine and taurine amidates plus sulphated steroids by gas–liquid chromatography using the method of Almé *et al.* (1977) as modified by Owen *et al.* (1984).

All legume seeds were analysed raw, and for this purpose a sample of haricot beans was obtained from the suppliers of the canned baked beans. Crude protein was determined by the Kjeldahl method no. 2 of Marshall & Walker (1978), and crude fat, ash and moisture by the Association of Official Analytical Chemists (1980) methods. Total, soluble and insoluble non-starch polysaccharides (NSP) were measured using the method of Englyst & Cummings (1988). Starch was calculated by difference. The seeds were also analysed for fatty acids by the International Union of Pure and Applied Chemistry: Commission on Oils, Fats and Derivatives (1979) method and for plant sterols (Owen *et al.* 1984).

Statistical methods

Statistical analysis was carried out using the Genstat 5 computer package. The data were analysed by analysis of variance (ANOVA), with pigs as blocks and diet crossed with linear and quadratic contrasts of period as the treatments. Where significant differences between treatments were established, individual differences between diet groups were tested by *t* test, with significance based on a probability of $P < 0{\cdot}01$.

Relationships between plasma lipids and faecal steroid excretion were analysed using Pearson's correlation coefficient, *r*.

RESULTS

General

All pigs remained in good health throughout the study and weight gains were satisfactory. Diet refusal was negligible and occurred only transiently on commencement of the experimental diets.

Plasma lipids

Fig. 1 depicts the changes in total plasma cholesterol that occurred during the 42 d experimental period.

Between-group differences in the rate of increase of total cholesterol were found to be significant overall using ANOVA (F 6·16, 15 and 90 df, $P < 0{\cdot}001$) and were mostly explained in terms of the linear trend (F 16·52, 5 and 90 df, $P < 0{\cdot}001$).

The semi-purified diet without added cholesterol (diet 1) induced a small but significant increase in plasma cholesterol from a basal mean of 2·5 mmol/l to 3·6 mmol/l ($P < 0{\cdot}01$) over the 42 d. This was caused by increases in both the HDL and VLDL + LDL fractions (Tables 2 and 3) which were evident after 14 d and did not increase further with time on the diet.

Supplementation of the semi-purified diet with 10 g crystalline cholesterol/kg (diet 2) provoked a large rise in total plasma cholesterol which was evident after 14 d and increased with time on the diet (Fig. 1). After 42 d the mean plasma cholesterol for the group was five times greater than its basal level ($P < 0{\cdot}001$) and more than three times greater than that for diet 1 at the same time-point ($P < 0{\cdot}001$). Most of the increase was associated with the VLDL + LDL fraction (Table 3), although HDL-cholesterol levels were also raised compared with basal levels ($P < 0{\cdot}01$, Table 2) and the elevation was greater than that observed for diet 1, suggesting that a proportion of the HDL increment was produced in response to the cholesterol supplement *per se*.

In general, substitution of legumes into the diet led to plasma cholesterol levels that were lower than those of control group 2 but higher than those of control group 1 (Fig. 1). Diet induced hypercholesterolaemia was significantly inhibited in all four legume groups compared with control group 2 (baked beans, peas and butter beans $P < 0{\cdot}001$, lentils $P < 0{\cdot}01$). Butter beans suppressed hypercholesterolaemia so effectively that the rate of increase of plasma cholesterol was not significantly different from that of control group 1 ($P > 0{\cdot}3$).

The differences in total cholesterol caused by the legumes in the diet were related principally to differences in the VLDL + LDL fraction. For all four groups the rate of increase in this fraction over time was significantly reduced compared with control group 2 (baked beans, peas and butter beans $P < 0{\cdot}001$, lentils $P < 0{\cdot}01$). In contrast, for the groups consuming baked beans, peas and lentils, HDL-cholesterol levels were not significantly different from those of control group 2. This suggests that the legume diets had a specific effect on the metabolism of the lower density lipoprotein fractions.

HDL:total cholesterol values (not shown) reflected the changes in cholesterol

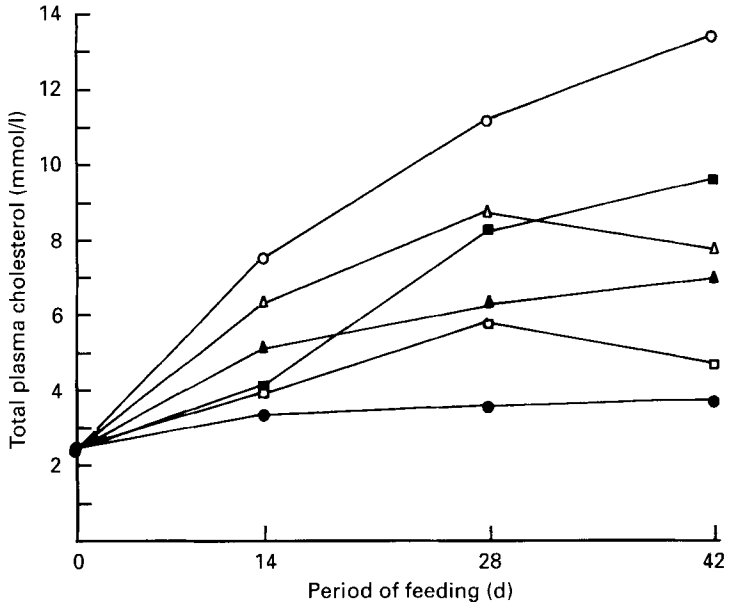

Fig. 1. Changes in mean plasma level of cholesterol of pigs consuming a hypercholesterolaemic semi-purified (SP) diet with or without substitution by one of four legume species. (●), Control diet 1 (SP); (○), control diet 2 (SP + 10 g cholesterol/kg); (▲), baked beans (*Phaseolus vulgaris*) (diet 3); (△), peas (*Pisum sativum*) (diet 4); (■), lentils (*Lens culinaris* Medik.) (diet 5); (□), butter beans (*Phaseolus lunatus*) (diet 6). Diets 3–6 contained SP + cooked legume (70:30, w/w) + 10 g cholesterol/kg. For details of diets and procedures, see Table 1 and pp. 410–411.

Table 2. *Mean values for high-density-lipoprotein (HDL)-cholesterol for four groups of pigs fed on different legume-containing diets and two control groups**

(Mean values and standard deviations)

Period of feeding (d)...	HDL cholesterol (mmol/l)								Statistical significance of difference: $P <$	
	0		14		28		42			
Dietary group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*v.* Diet 1	*v.* Diet 2
1 Control	0·85	0·09	1·61	0·22	1·48	0·20	1·55	0·20		0·01
2 Control	0·87	0·18	2·29	0·92	2·68	1·10	2·43	0·79	0·01	
3 Baked beans (*Phaseolus vulgaris*)	0·87	0·15	1·97	0·77	2·64	1·00	2·68	0·77	0·01	NS
4 Marrowfat peas (*Pisum sativum*)	0·86	0·17	2·17	1·01	2·52	0·84	2·32	0·55	0·01	NS
5 Red lentils (*Lens culinaris* Medik.)	0·74	0·07	1·69	0·55	2·49	0·58	2·38	0·67	NS	NS
6 Butter beans (*Phaseolus lunatus*)	0·82	0·15	1·54	0·39	1·89	0·34	1·85	0·28	NS	NS

NS, not significant.
Variance ratio F 2·16, $P = 0·086$.
* For details of diets and procedures, see Table 1 and pp. 410–411.

Table 3. *Mean values for very-low-density lipoprotein (VLDL)+low-density lipoprotein (LDL)-cholesterol for four groups of pigs fed on different legume-containing diets and two control groups**

(Mean values and standard deviations)

Period of feeding (d)...	VLDL-+LDL-cholesterol (mmol/l)								Statistical significance of difference: $P <$	
	0		14		28		42			
Dietary group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*v.* Diet 1	*v.* Diet 2
1 Control	1·62	0·37	1·74	0·46	2·04	0·19	2·03	0·12		0·001
2 Control	1·52	0·25	5·16	1·21	8·44	1·86	10·93	4·35	0·001	
3 Baked beans (*Phaseolus vulgaris*)	1·51	0·26	3·14	1·36	3·54	1·16	4·09	1·68	NS	0·001
4 Marrowfat peas (*Pisum sativum*)	1·64	0·49	4·13	2·04	6·03	4·16	5·43	2·55	0·01	0·001
5 Red lentils (*Lens culinaris* Medik.)	1·67	0·37	2·42	0·52	5·70	1·87	7·10	2·17	0·001	0·01
6 Butter beans (*Phaseolus lunatus*)	1·82	0·70	2·41	0·62	3·76	0·77	2·83	1·52	NS	0·001

NS, not significant.
Variance ratio F 11·41, $P < 0·001$
* For details of diets and procedures, see Table 1 and pp. 410–411.

concentration in the lipoprotein fractions. While the mean values at 42 d for control groups 1 and 2 were 0·43 and 0·19 respectively, comparable values for legume-based diets were 0·4, 0·33, 0·26 and 0·43 for baked beans, peas, lentils and butter beans respectively. ANOVA showed that only for lentils among the legume-based diets was the mean ratio significantly different ($P < 0·01$) from control group 1, and this was the only diet for which the mean ratio was not significantly different from control group 2.

Plasma triacylglycerol levels remained at basal level for all diets throughout the study.

Faecal steroids

Fig. 2 depicts the excretion of faecal neutral sterols, free bile acids and conjugated bile acids by each group during collection periods 1 and 2. The excretion of steroids for diet 1 during the first collection period averaged 41 mg/kg per d of which approximately half was in the form of neutral sterols (cholesterol, coprostanol and plant sterols). The remainder consisted mainly of free bile acids, of which hyodeoxycholic acid was the predominant species, with a small contribution coming from steroid amidates and sulphates. Overall excretion of steroid during period 2 was not different from that of period 1, although a greater percentage (76) of the total steroid occurred as neutral sterols.

In comparison with control group 1, control group 2 excreted significantly more neutral sterol (cholesterol and its bacterial conversion product coprostanol; $P < 0·001$) during both collection periods. However, a difference in the excretion of bile acids by the two groups was not apparent until period 2, when a small but significant increase in the production of each bile acid species was observed (values not shown). Steroid amidates and sulphates were not affected by the cholesterol supplement.

Each of the pigs receiving legume-substituted diets 3–6 showed the same response to the cholesterol supplement as those receiving diet 2, i.e. excretion of neutral sterols was significantly higher ($P < 0·001$) than that for the those receiving diet 1 during both collection periods, but an increase in the excretion of bile acids was not observed until

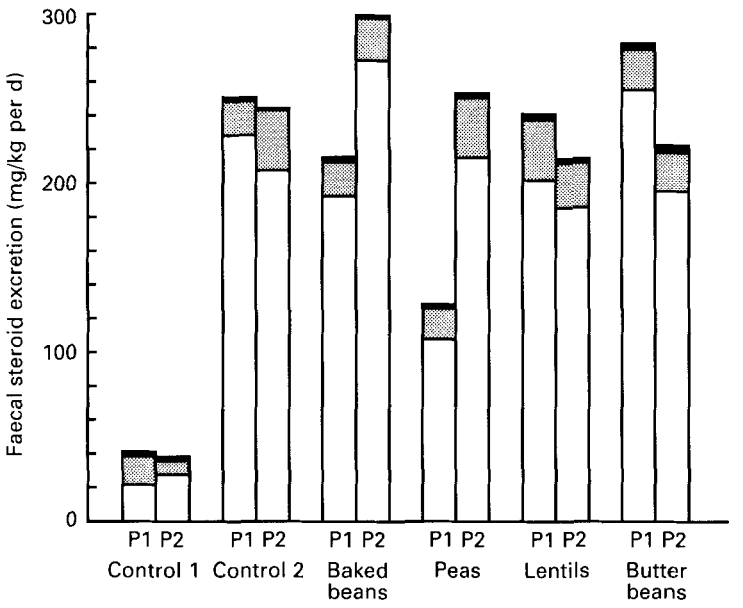

Fig. 2. Mean faecal steroid excretion of pigs consuming a hypercholesterolaemic semi-purified (SP) diet with or without substitution by one of four legume species during collection periods 1 (P1) and 2 (P2). Control diet 1, SP; control diet 2, SP + 10 g cholesterol/kg; diets containing legumes contained SP + cooked legume (70:30, w/w; respectively baked beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), lentils (*Lens culinaris* Medik.) or butter beans (*Phaseolus lunatus*)) + 10 g cholesterol/kg. (□), Total neutral sterols; (▨), total bile acids; (■), total steroid amidates and sulphates.

period 2. The amount of faecal steroid excreted by the pigs receiving baked beans, lentils and butter beans was not significantly different from that excreted by those given diet 2 during either period. Pigs receiving peas excreted significantly less neutral sterols than those receiving diet 2 during period 1 ($P < 0{\cdot}01$), but not during period 2.

Minor changes in the excretion of individual steroids occurred as a consequence of substituting legumes into the diet. The only change common to pigs receiving the legume-containing diets was an increase in the proportion of animal sterols occurring as coprostanol (a bacterial conversion product of cholesterol) compared with those given diet 2 (55 *v.* 38%, $P < 0{\cdot}01$ for all legume diets). This represented a return to values similar to those for diet 1 (values not shown).

Table 4 shows how much of the cholesterol provided in the diets of the five supplemented groups appeared in some form in the faeces. These values were obtained by calculating the amount of cholesterol fed to each animal on a particular day, and comparing this with the amount of steroid excreted in the faeces on the same day. The values assume no excretion of cholesterol resulting from endogenous synthesis. The percentage of dietary cholesterol excreted in the faeces varied widely within each group, but group means were similar. During period 1 the group consuming peas excreted less of their dietary cholesterol as steroid in the faeces than any other group, and this value was significantly different from control group 2 ($P < 0{\cdot}05$). Other than this there were no significant differences in steroid excretion between the diet groups.

Table 4. *Excreted steroid in the faeces of pigs receiving a hypercholesterolaemic diet with or without substitution by one of four legume species*†

(Mean values and standard deviations; values are expressed as a percentage of dietary cholesterol)

	Steroid (%)			
	Period 1		Period 2	
Dietary group	Mean	SD	Mean	SD
2 Control	83	28	81	14
3 Baked beans (*Phaseolus vulgaris*)	71	21	99	22
4 Peas (*Pisum sativum*)	42*	18	84	52
5 Lentils (*Lens culinaris* Medik.)	95	30	78	15
6 Butter beans (*Phaseolus lunatus*)	93	76	73	26

Mean value was significantly different from that for control diet 2: * $P < 0.05$.
† For details of diets and procedures, see Table 1 and pp. 410–411.

Table 5. *Composition of the four legume species used in the study*

(g/kg dry matter)

	Haricot bean (*Phaseolus vulgaris*)	Marrowfat pea (*Pisum sativum*)	Red lentil (*Lens culinaris* Medik.)	Butter bean (*Phaseolus lunatus*)
Crude protein (N × 6·25)	241	233	292	227
Crude fat	21	19	10	13
Starch (by difference)	489	569	627	578
Ash	39	26	19	32
Total NSP	188	153	52	150
Plant sterols (mg)	763	405	566	559

NSP, non-starch polysaccharide.

Relationships between plasma lipid levels and the excretion of faecal steroids

In general, plasma lipid levels were found to be unrelated to the excretion of steroids in the faeces. As assessed by Pearson's correlation coefficient, there were no significant relationships between plasma cholesterol level and the excretion of total steroids, total neutral steroids or total bile acids when the group was taken as a whole (n 36). During period 1 the excretion of cholesterol itself was positively related to the plasma cholesterol level at 14 d (r 0·359, $P < 0.05$). A similar relationship was observed when comparing cholesterol excretion during period 2 with the plasma cholesterol level at 42 d, but this did not quite reach statistical significance.

Amongst the pigs receiving the cholesterol supplement (n 30) there was a negative relationship between the plasma cholesterol level at 14 d and the excretion of lithocholic acid during period 1 ($r -0.521$, $P < 0.01$). The relationship was not significant if the values for diet 1 were included in the analysis. Paradoxically, during period 2 there was a positive

Table 6. *Composition of the non-starch polysaccharides (NSP) of the four legume species used in the study*

Sample	DM (g/kg)		Total (g/kg dry weight)	Composition (g/kg dry weight)								
					Monomeric constituents							
				Cellulose	RHA	FUC	ARA	XYL	MAN	GAL	GLU	U.AC
Haricot bean	864	Soluble NSP	67	—	1	3	27	6	3	8	t	19
(*Phaseolus vulgaris*)		Insoluble NSP	121	43	—	—	33	16	2	4	5	18
		Total NSP	188	43	1	3	60	22	5	12	6	36
Marrowfat pea	857	Soluble NSP	23	—	t	—	4	1	t	3	—	13
(*Pisum sativum*)		Insoluble NSP	131	59	1	—	32	12	t	5	4	17
		Total NSP	153	59	1	—	36	14	1	9	4	30
Red lentil	869	Soluble NSP	10	—	—	—	2	t	t	2	2	3
(*Lens culinaris* Medik.)		Insoluble NSP	42	9	—	—	22	3	t	3	1	6
		Total NSP	52	9	—	—	24	3	1	5	2	9
Butter beans	853	Soluble NSP	46	—	t	—	13	3	9	3	2	16
(*Phaseolus lunatus*)		Insoluble NSP	103	40	1	—	33	12	3	4	2	9
		Total NSP	150	40	1	—	47	16	12	6	3	25

—, none; ARA, arabinose; DM, dry matter; FUC, fucose; GAL, galactose; GLU, glucose; MAN, mannose; NSP, non-starch polysaccharide; RHA, rhamnose; t, trace; U.AC, uronic acid; XYL, xylose.

relationship between the excretion of lithocholic acid and the plasma cholesterol level at 42 d when data from all animals was included in the analysis (r 0·409, $P < 0·05$) but not when the analysis omitted diet 1. No other significant relationships were observed between plasma lipid levels and the excretion of faecal steroids.

Composition of the legumes

The proximate composition of each legume species is given in Table 5. Haricot beans, marrowfat peas and butter beans contained very similar amounts of protein, fat, starch and ash; red lentils contained lower levels of non-starch polysaccharides (NSP) and ash but proportionately more protein and starch owing to the removal of their hulls.

Crude fat (g/kg) in the legume seeds ranged from 10 for red lentils to 21 for haricot beans. The main fatty acids detected were palmitic ($C_{16:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) (values not shown). Haricot beans contained the highest level of polyunsaturates (72 g/100 g total fatty acids) and butter beans the highest level of saturates (29 g/100 g total fatty acids). There were large differences in the fatty acid profiles of the four legumes; however, it is unlikely that these were important determinants of plasma cholesterol levels because of the very small quantities of legume oil consumed by the animals. Plant sterols were also present in very small amounts (Table 5).

With the exception of lentils the legumes contained high levels of NSP which were predominantly insoluble and comprised largely cellulose plus arabinose- and xylose-based polymers (Table 6). The highest level of soluble NSP (67 g/kg) was found in haricot beans. Red lentils contained less than one-third of the NSP observed in the other legumes, but the composition of the NSP present was similar to that of the other species.

DISCUSSION

In the present study hypercholesterolaemia in pigs was induced by feeding a semi-purified diet supplemented with cholesterol. In response to this supplement the excretion of neutral sterols in the faeces was greatly increased. It is likely that this increase represented unabsorbed dietary sterol rather than an increase in hepatic synthesis; estimates of cholesterol absorption from the diet vary but it is thought that up to 80% of that presented to the gut mucosa is not absorbed by it (Grundy, 1983). In humans, excretion of cholesterol and coprostanol is increased on cholesterol feeding and most of the increase is due to unabsorbed dietary cholesterol (Quintao *et al.* 1971; Nestel & Poyser, 1976).

A significant increase in bile acid excretion in response to the cholesterol supplement was not seen until period 2, and when it did occur it was not large, amounting to only twice the excretion seen with the semi-purified diet alone. In some animal species increased bile acid excretion does appear to be a major compensatory mechanism for increased cholesterol absorption (Hulcher & Margolis, 1982). However, this has not been demonstrated conclusively in humans (Quintao *et al.* 1971; Nestel & Poyser, 1976), and the present study suggests that it is not a major defence mechanism against cholesterol overload in pigs.

Diet-induced hypercholesterolaemia in pigs was reduced when legumes were included as part of the diet. The observed reduction in plasma cholesterol was associated with a decrease in its concentration in circulating LDL, but did not appear to be associated with increased excretion of steroid in the faeces. These findings are similar to those of Anderson *et al.* (1984) who showed that humans consuming a controlled diet containing haricot beans as a source of soluble NSP experienced a reduction in plasma cholesterol, most of which occurred in the LDL fraction and which was not associated with an increase in faecal steroid excretion. The mechanism proposed by this group to explain the effect was that of reduced hepatic cholesterol synthesis mediated by propionate. The basis of this hypothesis

is that soluble NSP is fermented by the colonic flora with the production of propionic acid. This volatile fatty acid is readily absorbed and transported to the liver where it inhibits hydroxymethylglutaryl-CoA reductase (*EC* 1.1.1.88; HMG-CoA reductase), the rate-limiting enzyme of cholesterol synthesis. The reduced cholesterol synthesis is thought to be reflected in decreased plasma cholesterol levels.

In support of this hypothesis, the present study showed lentils, which contained the least total and soluble NSP, to be the least effective legumes with regard to cholesterol reduction. However, in spite of this it seems implausible that the propionate hypothesis can explain our results which were obtained under conditions of considerable cholesterol influx to the liver. Under these circumstances endogenous cholesterol synthesis would probably have been maximally inhibited by accumulation of endproduct, and it seems unlikely that further inhibition of hepatic synthesis by propionate would have had a measurable influence on plasma cholesterol levels. In future studies it may be helpful to measure the activity of HMG-CoA reductase in the liver to assess the extent of hepatic cholesterol synthesis.

Nevertheless, it is clear from the results of the present study and that of Anderson *et al.* (1984) that the steroid-binding hypothesis is not sufficient to explain the hypocholesterolaemic action of legumes in all cases. Indeed, the selective reduction of the LDL fractions in these and other studies suggests that legumes may instead have a specific effect on the metabolism of VLDL, intermediate-density lipoproteins (IDL) or LDL particles. Plasma lipoprotein levels are a measure of the interplay between supply by the liver and demand for their lipid constituents by the tissues. A reduction in plasma LDL therefore indicates either an increase in their catabolism or a reduction in their synthesis.

In an experiment designed to monitor the effect of baked beans on the turnover of LDL in pigs we observed that the fractional catabolic rate of ^{125}I-labelled LDL was the same in response to a hyperlipidaemic diet with or without baked beans, despite differences in the plasma cholesterol levels of the two groups (Shutler, 1988). Thus, baked beans appear to exert an effect on the synthesis of LDL by a mechanism not yet understood.

Selective lowering of LDL in the absence of increased faecal steroid excretion has also been observed in response to soya-bean protein (Kim *et al.* 1978; Noseda *et al.* 1980; Fumagalli *et al.* 1982), and this has led others to suggest that the effect is due to a reduced synthesis of LDL mediated by an altered balance of amino acids. The mechanism has not been clearly identified but two hypotheses are of interest. The limiting amino acid of soya bean and most other legumes is methionine, an essential amino acid which is required, among other things, for the formation of the methyl donor S-adenosyl methionine (SAM). SAM is functional in the synthesis of choline for phosphatidyl choline or lecithin, which is the principal phospholipid associated with LDL. This led Olson *et al.* (1958) to propose that the cholesterol-lowering effects of vegetable proteins could be the result of a relative deficiency of methionine or choline, or both, leading to limited numbers of LDL particles being available for the transport of cholesterol in the plasma.

This hypothesis would explain the observation of Terpstra *et al.* (1982) that the differential effects of animal and vegetable proteins only occur when the experimental animals or subjects are hypercholesterolaemic, since the quantities of methionine required would be directly related to the amount of cholesterol being processed by the liver. However, Kim *et al.* (1978) tested this hypothesis and found that supplementation of soya-bean protein with free methionine up to the level found in casein did not alter its hypocholesterolaemic properties when fed to hyperlipidaemic pigs. Similar results were obtained by Hamilton & Carroll (1976) in rabbits.

Legume proteins have a relatively low lysine: arginine value in comparison with animal proteins. This observation has received much attention from Kritchevsky *et al.* (1982) who

have demonstrated that the atherogenicity of a protein may be directly proportional to its lysine:arginine value. These authors showed that supplementing soya-bean protein with lysine to bring it up to the lysine:arginine value of casein raised plasma cholesterol levels in rabbits above those seen with soya-bean protein alone. Several mechanisms for the effect have been suggested, including stimulation of lipid absorption from the intestine by lysine, stimulation of glucagon release by arginine, and inhibition of hepatic arginase (*EC* 3.5.3.1) activity by lysine.

Analysis of the legumes used in the present study did not indicate any obvious component(s) which might have elicited the reduced plasma cholesterol levels seen.

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