Comparative effects on blood lipids and faecal steroids of five legume species incorporated into a semi-purified, hypercholesterolaemic rat diet

BY FATIMA D. DABAI, ANN F. WALKER*, IAN E. SAMBROOK, VERNON A. WELCH AND ROBERT W. OWEN

Department of Food Science and Technology, University of Reading, Whiteknights, Reading RG6 2AP

AND SAVITRI ABEYASEKERA

Department of Applied Statistics, University of Reading, Whiteknights, Reading RG6 2FN

(*Received 20 July 1994 – Revised 19 July 1995 – Accepted 7 August 1995*)

The cholesterolaemic effects in rats of a diet (VS) containing Bambara groundnuts (*Vigna subterranea*), a popular legume eaten in Nigeria, were compared with diets PV, PS, LC and PL, containing baked beans (*Phaseolus vulgaris*), marrowfat peas (*Pisum sativum*), lentils (*Lens culinaris* Medik.) and butter beans (*Phaseolus lunatus*) respectively. Sixty Sprague-Dawley rats were fed on hypercholesterolaemic semi-purified diets supplemented with 10 g cholesterol and 5 g cholic acid/kg and formulated to provide 40 % of energy from fat, as in a typical Western-type human diet. Legumes were substituted for 330 g/kg of the semi-purified diet on a dry-matter basis, which was modified to maintain the same contribution of energy sources as the control diet C3. Another ten rats were fed on control diet C2, which was similar to diet C3 but with no added cholesterol. The rats were fed for 8 weeks and plasma cholesterol levels were measured at weeks 4 and 8. The diets incorporating the five different legume species produced very different cholesterolaemic effects. Diets PV and PL were more potent at lowering raised plasma cholesterol levels than diets PS and LC. Inclusion of the Bambara groundnut into the semi-purified diet resulted in an exaggeration of hypercholesterolaemia. Differences in cholesterol-lowering capacity of the various legume diets in this experiment could not be related to concentrations of faecal bile acids or neutral sterols. However, there was evidence that the inclusion of legumes in the diets reduced the faecal excretion of secondary bile acids.

Hypocholesterolaemic effect: Steroids: Lipid metabolism: Legumes

Evidence for a hypocholesterolaemic effect of grain legumes on raised cholesterol levels in animal models and man has accumulated in recent years (Kingman, 1991). The mechanism for this is unknown, although various hypotheses have been promulgated. One favoured hypothesis is that the dietary fibre and/or saponins intervene in the enterohepatic circulation of bile acids and cholesterol, promoting enhanced faecal excretion of acidic and neutral steroids (Anderson & Chen, 1979; Oakenfull *et al.* 1979; Sidhu *et al.* 1987). However, while some studies have shown an increased excretion of bile acids as a result of legume feeding (Soni *et al.* 1982; Mahadevappa & Raina, 1983), others have not. Anderson *et al.* (1984) found that feeding *Phaseolus vulgaris* (haricot beans) led to a reduction in bile

* For reprints.

acid excretion in the faeces of human hypercholesterolaemic subjects. Kingman *et al.* (1993) found no difference in faecal steroid excretion in response to any of four separate legume species incorporated into diets fed to pigs compared with controls. In contrast, Costa *et al.* (1994) added baked beans (*Phaseolus vulgaris*) to a cholesterol-raising, semi-purified diet, fed to pigs, and found reduced bile acid excretion in the faeces, especially secondary bile acids, in comparison with control diets similarly formulated but without baked beans. These authors also analysed bile collected from the gallbladder of the animals at the end of the experiment and found that feeding baked beans increased the concentration of total bile acids in bile, particularly secondary bile acids. They proposed that a baked-bean-enriched diet enhances conservation of bile acids and cholesterol within the enterohepatic circulation. They considered that the high concentration of bile acids and cholesterol in bile may promote a feedback inhibition of hepatic cholesterol synthesis and therefore lead to a hypocholesterolaemic effect.

Although several legume species have been studied for their cholesterolaemic effects in separate experiments, there are few reports of comparative effects of different legume species added to a diet. For pigs fed on semi-purified diets supplemented with cholesterol (10 g/kg), Kingman *et al.* (1993) compared diets containing baked beans, marrowfat peas (*Pisum sativum*), red lentils (*Lens culinaris* Medik.) and butter beans (*Phaseolus lunatus*). Each diet was fed for 42 d. In comparison with a control diet also containing cholesterol, induced hypercholesterolaemia was significantly inhibited in the groups consuming baked beans, peas and butter beans, although HDL-cholesterol levels were maintained. While all legumes prevented the mean plasma concentrations of VLDL + LDL-cholesterol rising as much as a cholesterol-supplemented control, butter beans followed by baked beans were most potent, with red lentils and marrowfat peas showing less effect.

The objectives of the present study were (a) to investigate the cholesterol-lowering potential and effects on faecal steroid excretion of dietary Bambara groundnuts (*Vigna subterranea*) compared with other legumes for which these variables had previously been reported, and (b) to compare the more convenient rat model for the study of comparative cholesterolaemic effects of different legume species with the pig model previously used in this laboratory (Kingman *et al.* 1993). Thus the diets used by Kingman *et al.* (1993) were followed as closely as possible.

MATERIALS AND METHODS

Lentils (split, red) and butter beans were obtained from Whitworths, Wellingborough, Northants, marrowfat peas from Wherry & Sons, Bourne, Lincs. and Bambara groundnuts were purchased from Hong local government, in Adamawa State (formerly Gongola State), Nigeria. The baked beans were Gold Beans in tomato sauce (Richland Pure Food Co. Ltd, Malt House, Field End Rd, Eastcote, Middlesex) purchased in catering packs.

All dried legumes were cooked before compounding into diets. The seeds were soaked overnight at room temperature in excess deionized water, and then cooked in the soaking water in large, open, steam-jacketed pans. After cooking, the legumes were drained of cooking water, frozen in metal trays and freeze-dried. The baked beans were treated as the cooked beans above, except that during freeze-drying the sauce was included.

Animals and diets

Male Sprague-Dawley rats (*n* 80) weighing 124 (SD 10·2) g were obtained from Shaws Farm (Blackthorn, Oxon).

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

Ingredients	Diets							
	C1	C2	C3	PV	PS	LC	PL	VS
Rat chow	1000	—	—	—	—	—	—	—
Maize starch	—	383	383	281	201	214	218	236
Sucrose	—	84	84	16	74	75	68	80
Casein	—	157	157	70	70	56	66	52
Cellulose	—	60	60	—	—	—	—	—
Baked beans (*Phaseolus vulgaris*)	—	—	—	333	—	—	—	—
Marrowfat peas (*Pisum sativum*)	—	—	—	—	333	—	—	—
Red lentils (*Lens culinaris* Medik.)	—	—	—	—	—	333	—	—
Butter beans (*Phaseolus lunatus*)	—	—	—	—	—	—	333	—
Bambara groundnuts (*Vigna subterranea*)	—	—	—	—	—	—	—	333
Salt mixture*	—	41	41	41	41	41	41	41
Vitamin B complex†	—	40	40	40	40	40	40	40
Vitamin supplement‡	—	40	40	40	40	40	40	40
Vitamins ADE§	—	10	10	10	10	10	10	10
L-Cystine in starch‖	—	10	10	10	10	10	10	10
Soyabean oil	—	30	30	20	25	25	25	6
Tallow	—	145	145	136	155	156	149	150
Total	1000	1000	1000	1000	1000	1000	1000	1000
Cholesterol	—	—	10	10	10	10	10	10
Cholic acid	—	5	5	5	5	5	5	5

* Salt mixture comprised (g/kg): KH_2PO_4 379·1, $CaCO_3$ 371·8, NaCl 135·8, $MgCO_3.3H_2O$ 64·4, $FeSO_4.7H_2O$ 33·7, $MnSO_4.4H_2O$ 5·8, KI 0·8, $CuSO_4.5H_2O$ 0·4, $ZnSO_3$ 0·3, $CoCl_2$ 0·007, maize starch 7·0.

† The vitamin-B complex comprised (g/kg): cyanocobalamin 0·0008, biotin 0·005, inositol 5·49, *p*-aminobenzoic acid 2·49, pteroylmonoglutamic acid 0·38, maize starch 991·6.

‡ The vitamin supplement comprised (g/kg): thiamin 0·038, riboflavin 0·136, pyridoxine 0·63, ascorbic acid 0·107, pantothenic acid 0·628, menadione 0·033, nicotinamide 12·56, choline 73·07, maize starch 913·3.

§ The vitamins ADE mixture comprised (g/kg): cholecalciferol 0·040, DL-α-tocopheryl acetate 6·533, retinol 0·400, maize starch 993·0.

‖ L-Cystine in starch comprised (g/kg): L-cystine 150·0, maize starch 850·0.

Table 1 shows the composition of the experimental and control diets. Semi-purified diets were formulated such that, on addition of legumes, each diet provided the following (percentage total energy): fat 40, protein 12, carbohydrate 48, i.e. similar to the energy contributions of major constituents of a typical Western-type diet. For all diets the polyunsaturated fatty acids:saturated fatty acids (P:S) ratio was 0·2.

The legume-based diets and a semi-purified control diet (C3) were supplemented with 10 g cholesterol and 5 g cholic acid/kg to elevate blood cholesterol levels. There was a further control diet (C2), identical with C3 but without added cholesterol. Each diet was made up in a single batch of 15 kg. The cholesterol and cholic acid were thoroughly mixed in the soyabean oil and beef tallow before being added to the other ingredients and mixed thoroughly. The diets were pelleted and dried in a warm-air oven at 100°.

Experimental design

A total of seventy male Sprague-Dawley rats were randomly allocated to seven test groups of ten animals each, housed in pairs in solid-bottomed containers with wood shavings. A further ten animals fed only on rat chow were killed to provide baseline blood samples at the start of the experiment: blood samples were taken by heart puncture. The feeding period was 8 weeks. On arrival in the laboratory the animals were conditioned to their

surroundings for 3 d and fed on laboratory rat chow before commencing the experimental diets. All rats received feed and water *ad lib.*; individual rats were marked by ear-clipping. Amounts of food consumed and leftovers were recorded. Animals were weighed weekly and maintained under constant temperature and humidity in a room having a 12 h light–12 h dark cycle. The animals were started on their diets in five batches, each staggered by 1 week. At the start of each week, one cage containing two rats was set up for each experimental or control diet.

At week 4 of feeding, blood samples were taken by tail-vein bleeding, according to the technique of Conybeare *et al.* (1988). At the end of week 8 the animals were anaesthetized by intraperitoneal injection (Euthetal, May & Baker, Dagenham; 2 ml/kg body weight), the thoracic cavity was opened and blood samples taken by heart puncture. Blood samples were collected in heparinized syringes and tubes, shaken and transferred to centrifugation tubes and plasma was separated by centrifugation. The liver was removed, washed with saline (9 g NaCl/l) blotted to remove excess solution and blood, weighed and kept in a plastic bag at $-20°$ for liver lipid analysis.

In week 7, faeces from each cage were collected for 5 d for steroid analysis. Faeces were separated from urine as far as possible, placed in a suitable container, labelled and frozen. Before analysis, faeces were thawed, mixed thoroughly and a 10–15 g representative sample was freeze-dried. The freeze-dried sample was ground to a fine powder using a coffee grinder and stored in a sealed polythene bag at room temperature until required for analysis.

Analytical methods

Plasma was separated from the blood samples by centrifugation at 1500 ***g*** for 30 min at 4°. The separated plasma was aspirated and transferred to polycarbonate 'flip-top' containers, containing 2 μl 0·1 g/l sodium azide as preservative, according to the method of Chapman *et al.* (1981).

For the separation of HDL and LDL, the density of the plasma samples was adjusted to density (ρ) 1·05 g/ml with KBr and the samples were placed in balanced polycarbonate ultracentrifugation tubes. Each plasma sample was carefully overlayered with 2 ml ρ 1·05 g/ml KBr solution (prepared by diluting 12·223 ml ρ 1·21 g/ml KBr to 50 ml with water). The tubes of plasma were centrifuged in a Beckman L8 55 M ultracentrifuge (Beckman, High Wycombe, Bucks) for 20 h at 40000 rpm at 12°, using a Ti TLA-75 rotor, at minimum acceleration and deceleration. On removal of the tubes from the centrifuge, the top fraction ($4 \times 0·5$ ml) of the plasma solution (containing the LDL with small amounts of VLDL) was aspirated off using a curved pipette attached to a 10 ml plastic syringe, transferred to a 2 ml volumetric flask and made to the mark (LDL) with ρ 1·006 g/ml KBr solution. The sides of the ultracentrifuge tubes were carefully wiped with filter paper to remove remaining LDL. The bottom fraction remaining contained the HDL. This was transferred to a 5 ml volumetric flask using a straight pipette, sampling from the bottom of the tube until empty. The tube was washed with ρ 1·006 g/ml KBr solution (prepared by diluting 3·669 ml ρ 1·21 g/ml KBr to 100 ml with water). The washings were added to the volumetric flask to make up the volume to the mark. The LDL and HDL samples thus prepared were frozen at $-20°$ for cholesterol and triacylglycerol analyses.

Plasma HDL- and LDL-cholesterol and triacylglycerol were estimated using a fully automated clinical analyser (Baker ENCORE Clinical Chemistry System, Baker Instruments Ltd, Windsor, Berks.) in conjunction with an enzymic kit (ENCORE Reagent Set No. 27-012-700-000; Baker Instruments). The methods used were that of Allain *et al.* (1974), combined with the phenol–4 aminoantipyrine system of Trinder (1969) and Megraw *et al.* (1979). The total liver lipid was measured by the method of Folch *et al.* (1957), while

the method described by the International Union of Pure and Applied Chemistry (1979) was used for liver cholesterol.

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 is detailed by Costa *et al.* (1994). Results are reported as faecal concentration on a dry-weight basis, in the absence of weights for total faecal output.

Statistical analysis

Data for each variable studied were subjected to ANOVA to separate the overall variation due to different components, i.e., the differences between diets, between batches of animals (based on date of commencement of diet) and diet × batch interaction. The variation between diets was further split into components, to allow the following planned comparisons: (1) differences between control diets C2 and C3, (2) differences between legume diets, (3) differences between diet C2 and legume-based diets and (4) differences between diet C3 and legume-based diets. All data, except for values for C1, were included in the ANOVA. In cases where the ANOVA F test showed evidence of overall differences between diets, t tests were conducted corresponding to each of the above contrasts.

RESULTS

Animals

All animals remained healthy and active through to the 6th week of the 8-week experiment. However, by the 6th week, rats receiving Bambara groundnut (VS) and butter bean (PL) diets became notably less active. The total food intakes of the groups of rats (Table 2) during the experimental period of 8 weeks showed a significant difference ($P < 0{\cdot}01$) only between control diet C2 and the legume diets.

The mean weight gains of rats over the 8-week period are shown in Table 3. ANOVA followed by the t test showed that rats fed on the baked-bean diet (PV) weighed significantly less ($P < 0{\cdot}01$) than rats on all other diets, and that this was the major contributor to significant differences found in the ANOVA.

Plasma total cholesterol

The mean values for plasma cholesterol at weeks 4 and 8 for rats fed on control and legume diets are shown in Table 4. The mean value for rats at the beginning of the study (baseline control, C1) was lower than for rats in both the other control groups at weeks 4 and 8. However, the mean values for rats in control group C2 at both weeks 4 and 8 were significantly lower ($P < 0{\cdot}001$) than all other experimental groups. There were notable reductions in mean plasma cholesterol levels at week 8 in group C3 and all legume diet groups ($P < 0{\cdot}001$) compared with week 4.

At weeks 4 and 8, ANOVA (Table 4) revealed differences in mean plasma cholesterol levels of all diet groups tested: namely, between controls C2 and C3 ($P < 0{\cdot}001$), between legume diets ($P < 0{\cdot}001$), between C2 and legume diets ($P < 0{\cdot}001$) and between C3 and legume diets ($P < 0{\cdot}05$ and $P < 0{\cdot}001$ for weeks 4 and 8 respectively). The differences between mean values for rats fed on legume diets and control diet (C3) were further investigated using the t test. At weeks 4 and 8, the baked bean (PV) and the butter bean (PL) diets showed a significant effect ($P < 0{\cdot}01$ in each case) in preventing excessively raised plasma cholesterol levels, compared with diet C3. However, there was no significant difference between the marrowfat-pea (PS) diet or the lentil (LC) diet and C3. The rats fed

Table 2. *The mean weekly and total feed intake of rats on the experimental diets**

(Mean values for five groups of two rats caged in pairs, expressed on an individual rat basis)

Week ... Dietary group	1	2	3	4	5	6	7	9	Total feed intake (g)
C2 Control 2	259·0	270·9	327·2	285·1	335·2	309·3	309·0	286·5	2383
C3 Control 3	218·5	290·3	347·6	338·2	323·0	321·5	321·0	300·0	2443
PV Baked beans	298·7	325·0	440·0	370·2	340·0	325·1	300·0	286·0	2642
PS Marrowfat peas	290·6	312·7	398·7	379·5	336·7	325·6	341·0	335·2	2748
LC Red lentils	289·6	331·0	369·3	372·1	326·1	319·0	321·0	319·9	2648
PL Butter beans	290·0	345·0	389·1	362·1	341·7	356·1	319·0	327·1	2730
VS Bambara groundnuts	277·3	356·9	423·0	379·3	342·5	315·0	300·0	310·0	2704
Probability values for assessing specific comparisons between diet means (P):									
Difference between C2 and C3									NS
Difference between legume diets									NS
Difference between C2 and legume diets									< 0·01
Difference between C3 and legume diets									NS

* For details of diets and procedures, see Table 1 and pp. 558–560.

Table 3. *The body weight (g) of rats at the start of the experiment and weight gain after 8 weeks of feeding on experimental diets**

(Mean values and standard deviations for ten rats per dietary group)

	Starting wt (g)		Weight gain (g)	
Dietary group	Mean	SD	Mean	SD
C2 Control 2	124·2	10·5	334·0	10·5
C3 Control 3	125·0	17·8	336·9	17·8
PV Baked beans	127·2	11·8	270·2	11·9
PS Marrowfat peas	125·0	10·0	354·0	10·0
LC Red lentils	124·7	14·4	348·7	14·4
PL Butter beans	125·4	14·6	333·4	19·6
VS Bambara groundnuts	125·2	15·0	331·1	15·0
Probability values for assessing specific comparisons among diet means (P):				
Difference between C2 and C3			NS	
Difference between legume diets			< 0·001	
Difference between C2 and legume diets			< 0·001	
Difference between C3 and legume diets			< 0·001	

* For details of diets and procedures, see Table 1 and pp. 558–560.

on the Bambara-groundnut diet (VS) showed a mean plasma cholesterol value greater than that of rats fed on control diet C3, and the difference was significant in week 8 ($P < 0·01$).

HDL-cholesterol

The mean plasma HDL-cholesterol levels of the rats in the various diet groups at week 8 are also shown in Table 4. ANOVA showed that the HDL-cholesterol values were different ($P < 0·001$) for all the diet groups tested. The mean plasma HDL-cholesterol for rats in group C3 was not different compared with marrowfat pea (PS) and lentil (LC) diets, but was less than values for groups VS, PL and PV ($P < 0·01$).

Table 4. *Mean plasma total cholesterol concentrations of rats fed on experimental diets for 4 and 8 weeks and mean cholesterol contents of plasma HDL and LDL and HDL: total cholesterol ratios at 8 weeks, with baseline control (C1)**

(Mean values and standard deviations for ten rats per dietary group)

	Plasma cholesterol (mmol/l)								HDL: total cholesterol ratio at 8 weeks	
	Total at 4 weeks		Total at 8 weeks		HDL at 8 weeks		LDL at 8 weeks			
Dietary group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C1 Control 1 (Baseline, week 0)	2·24	0·09	—	—	0·54	0·06	0·32	0·02	0·24	0·02
C2 Control 2	2·71	0·15	3·13	0·23	0·64	0·08	0·91	1·27	0·50	0·04
C3 Control 3	10·34	0·72	6·72	0·60	0·98	0·12	4·87	1·27	0·15	0·02
PV Baked beans	7·06	0·29	4·28	0·32	1·39	0·09	2·29	1·38	0·33	0·03
PS Marrowfat peas	8·68	1·02	5·78	0·65	0·90	0·06	3·56	1·38	0·18	0·02
LC Red lentils	9·72	1·22	6·27	0·42	0·96	0·05	3·30	0·70	0·16	0·01
PL Butter beans	8·48	0·69	5·11	0·42	1·38	0·07	2·98	1·68	0·30	0·04
VS Bambara groundnuts	12·02	1·39	8·66	0·91	1·65	0·11	4·70	1·11	0·21	0·04
Probability values for assessing specific comparisons among diet means (P):										
Difference between C2 and C3	< 0·001		< 0·001		< 0·001		< 0·001		< 0·001	
Difference between legume diets	< 0·001		< 0·001		< 0·001		< 0·001		< 0·001	
Difference between C2 and legume diets	< 0·001		< 0·001		< 0·001		< 0·001		< 0·001	
Difference between C3 and legume diets	< 0·05		< 0·001		< 0·001		< 0·001		< 0·001	

* For details of diets and procedures, see Table 1 and pp. 558–561.

The mean HDL: total cholesterol ratios are shown in Table 4 for all diet groups, together with data for rats fed on diet C1. Significant differences ($P < 0·001$) were observed for all the diets when tested by ANOVA. However, the t test showed no significant difference in mean ratios between rats on diet C3 and those fed on diets LC or PS, but diets PV and PL yielded ratios significantly higher than those for rats fed on diet C3 ($P < 0·01$).

LDL-cholesterol

The mean plasma LDL-cholesterol levels for the various diet groups at week 8 are shown in Table 4. The ANOVA showed differences in all diet groups tested ($P < 0·001$). The t test showed that the mean plasma LDL-cholesterol values for all the diet groups were significantly reduced compared with those fed on C3, except for the rats fed on the Bambara-groundnut (VS) diet, which showed no difference.

Triacylglycerol

Table 5 shows the mean values of plasma triacylglycerols of rats at weeks 4 and 8. The mean plasma triacylglycerol levels of rats fed on both C2 and C3 diets at week 4 were greater ($P < 0·001$) than corresponding values at week 8. At week 4 the mean plasma triacylglycerol level of group C2 was greater than that of group C3 ($P < 0·001$) and those of rats fed on legume diets ($P < 0·001$). At week 8, although a similar pattern was observed, all the values were reduced compared with week 4. Mean values of HDL- and LDL-triacylglycerol for the various experimental groups in week 8 are shown in Table 5. ANOVA revealed that the differences in plasma triacylglycerol appeared to be due to differences in LDL-triacylglycerol rather than HDL-triacylglycerol.

Table 5. *Mean plasma triacylglycerol concentrations of rats fed on experimental diets for 4 and 8 weeks and mean triacylglycerol contents of plasma HDL and LDL at 8 weeks, with baseline control (C1)**

(Mean values and standard deviations for ten rats per dietary group)

	Triacylglycerol (mmol/l)							
	Total at 4 weeks		Total at 8 weeks		HDL at 8 weeks		LDL at 8 weeks	
Dietary group	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C1 Control 1 (Baseline, week 0)	0·650	0·041	—	—	0·026	0·030	0·281	0·025
C2 Control 2	2·387	0·238	1·540	0·089	0·174	0·020	1·066	0·152
C3 Control 3	1·365	0·165	0·818	0·111	0·175	0·017	0·492	0·080
PV Baked beans	0·730	0·059	0·498	0·052	0·163	0·027	0·239	0·063
PS Marrowfat peas	0·959	0·149	0·725	0·090	0·166	0·017	0·387	0·041
LC Red lentils	0·839	0·083	0·546	0·045	0·114	0·017	0·367	0·047
PL Butter beans	1·022	0·132	0·795	0·081	0·142	0·015	0·484	0·040
VS Bambara groundnuts	0·673	0·085	0·531	0·670	0·152	0·018	0·260	0·042
Probability values for assessing specific comparisons between diet means (P):								
Difference between C2 and C3	< 0·001		< 0·001		NS		< 0·001	
Difference between legume diets	NS		NS		NS		NS	
Difference between C2 and legume diets	< 0·001		< 0·001		NS		< 0·001	
Difference between C3 and legume diets	NS		NS		NS		NS	

* For details of diets and procedures, see Table 1 and pp. 558–561.

Liver weight

Table 6 shows the mean liver weights of rats fed on control and experimental diets. The livers of rats fed on diet C2 were of normal size and a healthy dark colour at week 8, while those of group C3 and rats fed on the cholesterol-supplemented, high-fat legume diets were swollen and pale in appearance. ANOVA revealed lower mean weights for rats in group C2 compared with those fed on diets C3 and the legume diets ($P < 0·001$ in each case) and this was reflected in comparisons of the mean liver:body-weight ratios.

Liver lipid

The values of mean percentage liver lipid and its cholesterol content are shown in Table 6. ANOVA showed differences between values for rats fed on diet C2 compared with C3 ($P < 0·001$) and between each of these and the legume diets ($P < 0·001$ and $P < 0·01$ respectively), but not between legume diets. The mean values for cholesterol in liver lipid are also shown in Table 6. ANOVA showed significantly lower values for rats fed on diet C2 compared with diet C3, between diet C2 and the legume diets ($P < 0·001$), and between legumes ($P < 0·05$) but none between rats fed on diet C3 and legume diets. The t test showed that the group fed on the cholesterol-supplemented diet containing Bambara groundnut (VS) resulted in a higher mean value for liver cholesterol than that for the group fed on diet C3 ($P < 0·05$).

Faecal steroids

The mean values for bile acids and neutral steroids present in the faeces of the rat groups (faeces collected from pairs of animals housed together) are given in Table 7 and the probability values for assessing specific comparisons of these values for the different diet

Table 6. *Liver fresh weight and lipid composition, liver: body weight and cholesterol content of liver lipid of rats fed on experimental and control diets for 8 weeks, with baseline control (C1)**

(Mean values and standard deviations for ten rats per dietary group)

Rat group	Liver wt (g)		Liver: body-wt ratio ($\times 10^2$)		Total lipid (mg/g)		Cholesterol (mg/g total lipid)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C1 Control 1	6·6	0·3	5·20	0·4	42	3	10	0·1
C2 Control 2	17·8	0·6	3·93	0·1	72	5	6	1·0
C3 Control 3	27·9	1·3	6·23	0·1	278	11	93	4·0
PV Baked beans	25·3	1·4	6·24	0·2	287	8	109	6·0
PS Marrowfat peas	28·8	1·3	6·18	0·2	276	9	98	4·0
LC Red lentils	29·1	1·4	6·29	0·2	284	12	107	6·0
PL Butter beans	28·6	1·5	6·42	0·2	268	17	87	6·0
VS Bambara groundnuts	30·2	1·7	6·93	0·3	300	9	113	9·0
Probability values for assessing specific comparisons among diet means (P):								
Difference between C2 and C3	< 0·001		< 0·001		< 0·001		< 0·001	
Difference between legume diets	NS		NS		NS		< 0·001	
Difference between C2 and legume diets	< 0·001		< 0·001		< 0·001		< 0·001	
Difference between C3 and legume diets	NS		NS		NS		NS	

* For details of diets and procedures, see Table 1 and pp. 558–561.

Table 7. *Faecal output, on a dry matter basis (mg/g), of neutral and acidic steroids of rats fed on experimental and control diets for 8 weeks**

(Mean values and standard deviations for five pairs of rats per dietary group)

Diet...	C2		C3		PV		PS		LC		PL		VS	
Steroid	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cholesterol	4·7	1·4	31·1	5·2	32·7	6·6	40·1	5·6	44·0	4·5	29·3	1·3	39·8	3·1
Coprostanol	0·3	1·0	5·3	0·9	5·0	1·6	1·9	0·6	3·1	1·2	4·2	1·4	6·3	1·3
Total animal steroids	5·1	1·5	36·4	5·3	37·0	5·6	42·0	6·2	47·1	4·0	33·2	1·3	47·3	0·8
Total plant steroids	2·1	7·8	7·2	2·4	11·3	5·8	14·2	3·0	13·5	4·5	10·3	4·7	9·2	2·3
Total neutral steroids	7·1	2·2	43·6	6·5	49·0	5·6	56·2	4·9	60·6	6·5	43·5	2·4	56·5	5·2
Coprostanol of TNS (%)	4·1	—	12·3	—	10·2	—	3·4	—	5·1	—	9·7	—	11·4	—
Cholic acid	5·9	2·6	10·6	1·8	20·0	2·6	15·5	3·2	23·1	2·6	13·5	2·6	26·3	0·7
Chenodeoxycholic acid	1·8	1·5	1·0	0·3	1·6	0·2	1·5	0·4	1·9	0·4	1·7	0·5	1·9	0·4
Deoxycholic acid	14·7	2·2	14·1	1·3	9·7	2·1	6·6	2·8	7·8	1·4	11·7	2·0	4·8	1·0
TFBA	22·4	—	25·8	—	31·3	—	33·6	—	32·8	—	26·9	—	32·9	—
Primary of TFBA (%)	34·0	—	45·0	—	69·0	—	50·6	—	76·2	—	56·5	—	85·7	—
Sulphated conjugated bile acid	0·3	0·05	0·6	0·22	0·6	0·2	0·4	0·1	0·4	0·1	0·3	0·1	0·4	0·1

TFBA, total faecal bile acids; TNS, total neutral steroids.

* For details of diets and procedures, see Table 1 and pp. 558–561.

groups are shown in Table 8. The concentration of cholesterol in the faeces (dry-matter basis) was much less in rats fed on control diet C2 compared with control diet C3 or compared with legume diets ($P < 0·001$). However, there were no differences between values for rats fed on the legume diets or between those fed on C3 and the legume diets.

Table 8. *The probability values from ANOVA for assessing specific comparisons of faecal steroids of rats fed on experimental and control diets for 8 weeks**

Variables	(1)	(2)	(3)	(4)
Cholesterol	< 0·001	NS	< 0·001	NS
Coprostanol	< 0·001	NS	< 0·001	< 0·05
Total animal steroids	< 0·001	< 0·001	< 0·001	NS
Total plant steroids	NS	< 0·001	< 0·001	NS
Total neutral steroids (TNS)	< 0·001	< 0·001	< 0·001	NS
Coprostanol of TNS (%)	< 0·05	NS	NS	NS
Cholic acid	NS	< 0·001	< 0·001	< 0·001
Chenodeoxycholic acid	NS	NS	NS	NS
Deoxycholic acid	NS	NS	< 0·001	< 0·05
Sulphated conjugated bile acid	< 0·05	NS	NS	NS

(1), Differences between controls C2 and C3; (2), differences between legume diets; (3), differences between C2 and legume diets; (4), differences between C3 and legume diets.

* For details of diets and procedures, see Table 1 and pp. 558–561.

Mean faecal coprostanol concentrations were much lower for the group of rats fed on diet C2 than for all the other diets tested ($P < 0·001$) and values for rats fed on diet C3 were higher than for the legume diets ($P < 0·05$). Although there was much variability in the mean values for this metabolite between the different legume diets, this was not found to be significant because of data variability.

Total animal sterols is the sum of cholesterol and coprostanol concentrations, and besides showing similar trends to cholesterol values, the difference between mean values for animals fed on different legume diets was significant ($P < 0·001$): values for rats fed on diets PV (baked beans) and PL (butter beans) were lower than for the other legume diets.

Total plant sterols concentration was not different between diets C2 and C3, which would be expected as the diets were very similar, but there were differences in concentration between the legumes ($P < 0·001$). Although the variability for some of the mean data was large, concentration values for rats fed on different legume diets ranged from a mean value of 9·2 (VS) to 14·2 (PS) mg/g dry weight.

Mean faecal cholic acid concentrations of rat groups fed on the two control diets were not significantly different, but differences were found between the legume diets ($P < 0·001$), with mean values ranging from 13·5 mg/g (diet PL) to 26·3 mg/g (diet VS). Mean faecal cholic acid concentrations for rats fed on the legume diets were significantly higher than those for groups C2 and C3 ($P < 0·001$ in both cases).

While there was no difference in the mean values for faecal concentration of the primary bile acid chenodeoxycholic acid between any of the diet groups tested (Table 8), the concentration of the secondary bile acid deoxycholic acid was lower on the legume diets than in either rat groups C2 ($P < 0·001$) or C3 ($P < 0·05$). Thus, for all legume-diet groups, although the faecal total free bile acid concentration was very similar to both controls, the percentage occurring as primary bile acids was higher than for either control.

DISCUSSION

Body weight

The reason for lower weight gains of rats fed on the baked-bean diet (PV) compared with the other experimental diets, including the controls, is not clear, particularly as no difference in feed intake over the 8-week study period was noted between legume-based

diets or between legume-based diets and C3. The effect of a bean (*Phaseolus vulgaris*) diet on weight gain compared with control was noted by Chang *et al.* (1986) in rats, by Costa *et al.* (1993, 1994) in pigs, and by Anderson *et al.* (1990) in human subjects. However this is the first report of differences in weight gain of rats fed on different legume species. This unexpected reduction in weight gain due to the inclusion of baked beans in the diet compared with other legumes deserves further investigation.

Plasma cholesterol

The decline in plasma cholesterol levels at week 8 compared with week 4 in all experimental groups suggests an adaptation to the diets. However, this did not interfere with the ranking order of the treatments. Adaptation and even restoration of plasma lipid levels has been observed in human experiments of sufficient duration (Edgington *et al.* 1987).

The inclusion of 300 g freeze-dried legumes/kg in the semi-purified, high-fat and high-cholesterol diets resulted in significant reductions in the mean values of plasma cholesterol from the diet groups compared with those fed on control diet C3 (Table 4). The greatest reductions were for rats fed on the baked-bean diet (PV) and butter-bean diet (PL). Although there was a reduced weight gain of the group of rats fed on diet PV, this was not true for rats fed on the PL diet, so it is unlikely that differences in weight gain would account for the hypocholesterolaemic effect of diet PV.

It is apparent from the results presented here and from those reported by other investigators (e.g. Anderson *et al.* 1984) that some legumes have a cholesterol-lowering effect, but they may not all be equally potent. Soni *et al.* (1979) reported that lentils had no effect on reducing plasma cholesterol in rats, while peas increased the level. However, the experimental conditions were very different from those chosen here: rats were fed on low-fat diets with no added cholesterol. Kingman *et al.* (1993) carried out an experiment with pigs fed on similar diets to those used here, except that the diets were not supplemented with cholic acid. They reported that diet-induced hypercholesterolaemia was significantly inhibited by diets containing baked beans, peas, butter beans and lentils, but that butter beans and baked beans were the most effective at reducing LDL-cholesterol, which accords with our findings. An unexpected finding from the present study was that rats fed on the Bambara-groundnut diet (VS) showed plasma cholesterol values in week 8 which were significantly higher than values for rats fed on diet C3 ($P < 0{\cdot}01$). This indicates that Bambara groundnut is unusual among legumes reported on thus far, in causing a rise in plasma cholesterol in a hypercholesterolaemic animal model.

It was notable that the reductions in plasma cholesterol level observed with diets PV and PL were accompanied by a significantly higher HDL-cholesterol:total cholesterol ratio, compared with the other legume diets. In man at least, a high ratio is considered to reduce risk of coronary heart disease, even when total cholesterol level is elevated, although the significance of the ratio in the rat is less clear, as most cholesterol is transported in HDL in this species. (It should be noted that in the data presented in Table 4 the HDL:total cholesterol ratio is less than 0·5 for all treatments. Since most of the circulating cholesterol is carried by HDL in the rat, this would suggest that there was less than 100% recovery of plasma cholesterol in the HDL fraction.) There is supporting evidence from studies in pigs (Kingman *et al.* 1993) that the ratio is preserved by these two legume species. In addition, there is evidence in man (Shutler *et al.* 1989) that baked beans preserve a high ratio.

Many dietary factors have been reported to contribute to the hypocholesterolaemic effects of dietary legumes. In the present study the following possibilities were eliminated by the use of standardized diets: total fat content, polyunsaturated fatty acid content of lipid compared with saturated fatty acid content (P:S ratio) and protein content. Like Kingman *et al.* (1993), we have not been able to eliminate the possibility of the involvement

of NSP. Much of the NSP in legume seeds is present in soluble form. Lin & Anderson (1978) showed that guar gum, an isolated soluble-NSP fraction prepared from the Indian cluster bean (*Cyamopsis tetragonoloba*), is hypocholesterolaemic when fed to rats with raised cholesterol levels, in contrast to insoluble NSP from cereals which has no effect.

Using the method of Englyst & Cummings (1988), Kingman *et al.* (1993) reported the soluble NSP of baked beans (haricot variety), marrowfat peas, red lentils and butter beans to be 67, 23, 10 and 46 g/kg DM respectively. Using the same methodology, Dabai (1992) reported that Bambara groundnuts contain 33 g soluble NSP/kg DM. It was notable that the two species showing greatest hypocholesterolaemic effects, namely baked beans and butter beans, are also highest in soluble NSP. The smaller soluble-NSP content of Bambara groundnut might have contributed to its inability to lower raised cholesterol levels, but is unlikely to account for the cholesterol-raising effect of this legume, which was not shown by lentils and peas which have contents of soluble NSP lower than that of the Bambara groundnut. These findings indicate that, even if soluble NSP is involved, other factors may also moderate the cholesterol-lowering response. For example, minor components such as saponins have also been reported to have cholesterol-lowering effects (Oakenfull *et al.* 1979; Sharma, 1987).

Liver weight and lipid content

The large liver weights observed at the end of the study in rats fed on the control diet C3 and legume diets can be attributed to the supplement of the cholesterol with cholic acid added to the high-fat diet. Feeding a high-fat, high-cholic-acid diet without additional cholesterol did not result in increased liver weight (C2). Prema & Kurup (1973) and Beynen *et al.* (1984, 1986) also observed heavy liver weights of rats fed on high-fat, high-cholesterol diets.

All the heavy livers were very pale in colour and contained much greater proportions of total lipid and cholesterol than those from rats fed on diet C2, again agreeing with the observations of Beynen *et al.* (1984). It had previously been noted that by the 6th week of the experiment some groups of rats were less active and this was most marked in those with largest livers at the end of the experiment, suggesting that accumulation of fat in the liver of the animals had induced liver dysfunction. Thus, although the dietary regimen used here for raising blood cholesterol in the rat has been the most extensively used by other workers, the severe pathological changes that it induced would indicate that the model is far from ideal.

Faecal steroids

Mean coprostanol concentrations were lower in the faeces of the animals fed on legume diets than for the group fed on diet C3, except for rats fed on diet VS. Coprostanol is a metabolite of cholesterol, formed by the action of the microflora in the gut, and its presence may indicate fermentation activity in the large intestine. Costa *et al.* (1994) showed that pigs fed on a baked-bean diet similar to that fed here with the supplementation of cholesterol (but no cholic acid) showed a higher excretion of coprostanol compared with pigs fed on a similar diet, in which fermentation in the large intestine had been eliminated by an ileo-rectal anastomosis, or compared with intact pigs fed on a semi-purified control diet, low in soluble NSP.

The presence of large amounts of coprostanol in the faeces may indicate the extent of bacterial fermentation in the large gut, or the time of faecal residence in the gut. The presence of soluble NSP might be associated with a high coprostanol content of the faeces, but so would the presence of residual starch, which is also fermentable and was not measured in the present study. Although the discovery of coprostanol in the faeces of rats fed on legume diets may be due to the presence of soluble NSP, this cannot be so for the

high level in the faeces of rats fed on diet C3. This control contained no soluble fibre, but the unintentional presence of residual starch cannot be ruled out. The habit of coprophagy in the rat makes interpretation of the consequences of microbial fermentation difficult.

The animals fed on the legume diets showed higher concentrations of plant sterols in faeces (dry-matter basis) when compared with those fed on C2. Dietary plant sterols have been suggested to inhibit cholesterol absorption by competing for mucosal binding sites. Although these data give no indication of the compositional profile of the plant sterols, there were significant differences between the total mean plant sterol concentrations in faeces of rats fed on the different legume diets. On the basis of these results, it seems unlikely that total plant sterols are responsible for the more potent hypocholesterolaemic effects of baked beans and butter beans, as the faeces of rats on these diets showed lower plant sterol content than those on the pea and lentil diets, which showed less potent effect.

Although all experimental diets and controls contained the same amount of cholic acid, the concentrations in the faeces of rats fed on legume-based diets were higher than for either control group. These findings are in agreement with the study of Soni *et al.* (1982) who observed higher cholic acid concentrations in faeces of rats fed on cholic-acid-supplemented legume-based diets compared with cholic-acid-supplemented controls. The enhanced cholic acid concentration may indicate cholic acid binding to components of the legume diets (such as dietary fibre).

The faecal concentration of the secondary bile acid deoxycholic acid (recycled in the enterohepatic circulation) was lower for all the legume diets than either control diet which might indicate that there were fewer primary bile acids available for absorption in the legume-supplemented diets. Costa *et al.* (1994) also reported a reduction in secondary bile acid excretion in pigs fed on baked beans compared with a control diet. These authors also collected bile samples from the gall bladders of the animals at slaughter, which showed a higher concentration of bile acids, especially secondary bile acids, in those pigs fed on the baked-bean diet compared with control. These authors 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. They suggested that the high concentration of bile acids (and cholesterol) in bile promotes a feedback inhibition of hepatic cholesterol synthesis and thus reduces plasma cholesterol. The reduced concentration of secondary bile acids in the rat faeces in the present study would accord with this, but there was no further support for this hypothesis from the current study, due to the differences (dietary, hepatic, and gastrointestinal) in the hypercholesterolaemic model used.

CONCLUSIONS

The inclusion of the seeds of five different legume species in the diets of rats supplemented with cholesterol and cholic acid (10 g/kg and 5 g/kg respectively on a dry-matter basis) produced very different cholesterolaemic effects. Baked beans and butter beans were more potent at lowering raised plasma cholesterol levels than marrowfat peas and lentils. Inclusion of the Bambara groundnut resulted in an exaggeration of hypercholesterolaemia.

Despite the fact that the rat model showed cholesterolaemic differences between different legume-based diets, the addition of cholesterol and cholic acid to the diet resulted in severe liver pathology. There is a need for the model to be re-examined before being used further to assess the cholesterolaemic effects of foods.

Differences in cholesterol-lowering capacity of the various legume diets in this experiment could not be related to faecal bile acid concentration, or concentration of neutral sterols. However, there was evidence that the inclusion of legumes in the diets reduced the faecal concentration of secondary bile acids.

We are grateful to the Adamawa (formerly Gongola) State Government of Nigeria for providing a scholarship to FDD. The authors wish to thank Mrs S. E. Lane, Mrs J. B. Barley and Mr H. A. Smith for their excellent technical assistance and Mr J. D. Edwards-Webb for advice on animal diets. Thanks are also due to Mr I. M. Wilson for statistical advice and to Whitworths (Wellingborough, Northants) for the gift of lentils and butter beans.

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.

Beynen, A. C., Lemmens, A. G., De Bruije, J. J., Katan, M. B. & Nab Zutphen, L. F. M. (1986). Interaction of dietary cholesterol with cholate in rats: effect of serum cholesterol, liver cholesterol and liver function. *Nutrition Reports International* **34**, 557–563.

Beynen, A. C., Weinans, G. J. B. & Katan, M. B. (1984). Arylesterase activities in the plasma of rats, rabbits and humans on low- and high-cholesterol diets. *Comparative Biochemistry and Physiology* **78**, 669–673.

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

Chapman, M. J., Goldstein, S., Lagrane, D. & Lapland, P. M. (1981). A density gradient ultracentrifugal procedure for the isolation of major lipoprotein classes from human serum. *Journal of Lipid Research* **22**, 339–358.

Conybeare, G., Leslie, G. B., Angles, K., Barrett, R. J., Luke, J. S. & Gask, D. R. (1988). An improved simple technique for the collection of blood samples from rats and mice. *Laboratory Animals* **22**, 177–182.

Costa, N. M. B., Low, A. G., Walker, A. F., Owen, R. W. & Englyst, H. N. (1994). Effect of baked beans (*Phaseolus vulgaris*) on steroid metabolism and non-starch polysaccharide output of hypercholesterolaemic pigs with or without an ileo-rectal anastomosis. *British Journal of Nutrition* **71**, 871–886.

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

Dabai, F. D. (1992). Nutritional and technological properties of Bambara groundnut in infant and adult food. PhD Thesis, University of Reading.

Edgington, J., Gecki, M., Carter, R., Benfield, L., Fisher, K., Ball, M. & Mann, J. (1987). Effects of dietary cholesterol on plasma cholesterol concentrations in subjects following reduced fat, high fibre diet. *British Medical Journal* **294**, 333–336.

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

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

International Union of Pure and Applied Chemistry (1979). *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 6th ed., pp. 96–98 [C. Paquot, editor]. Oxford: Pergamon Press.

Kingman, S. M. (1991). The influence of legume seeds on human plasma lipid concentrations. *Nutrition Research Reviews* **4**, 97–123.

Kingman, S. M., Walker, A. F., Low, A. G., Sambrook, I. E., Owen, R. W. & Cole, T. (1993). Comparative effects of four legume species on plasma lipids and faecal steroid excretion in hypercholesterolaemic pigs. *British Journal of Nutrition* **69**, 409–421.

Lin, W. J. & Anderson, J. W. (1978). Effects of guar gum and wheat bran on lipid metabolism in rats. *Federation Proceedings* **37**, 542.

Mahadevappa, V. G. & Raina, P. L. (1983). Hypocholesterolaemic effect of cowpea in rats on atherogenic diet. *Indian Journal of Medical Research* **78**, 819–823.

Megraw, R. E., Dunn, D. E. & Biggs, H. E. (1979). Manual and continuous flow colorimetry of triacylglycerols by a fully enzymic method. *Clinical Chemistry* **25**, 273–278.

Oakenfull, D. G., Fenwick, D. E., Hood, R. L., Topping, D. L., Illman, J. R. & 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.

Prema, L. & Kurup, P. A. (1973). Effect of protein fractions from red gram (*Cajanus cajan*) and horse gram (*Dolichos biflorus*) on the serum, liver and aortic lipid levels in rats fed a high-fat–high-cholesterol diet. *Atherosclerosis* **18**, 369–377.

Sharma, R. D. (1987). An evaluation of hypocholesterolaemic activity of some uncommon legumes. *Nutrition Research* **7**, 351–363.

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, normocholesterolaemic men. *British Journal of Nutrition* **61**, 257–265.

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.

Soni, G. I., George, M. & Singh, R. (1982). Role of common Indian pulses as hypocholesterolaemic agents. *Indian Journal of Nutrition and Dietetics* **19**, 184–190.

Soni, G. I., Sohal, B. S. & Rattan, S. (1979). Comparative effect of pulses on tissue and plasma cholesterol levels in albino rats. *Indian Journal of Biochemistry and Biophysics* **16**, 444–446.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry* **6**, 24–27.

Printed in Great Britain