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Local (gut) and systemic metabolism of rats is altered by consumption of raw bean (*Phaseolus vulgaris* L. var. *athropurpurea*)

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The composition of the raw legume *Phaseolus vulgaris* L. var. *athropurpurea* (PhVa) and its effects on the metabolism of young growing rats have been evaluated. The levels of protein, unsaturated fatty acids, carbohydrate, fibre and bioactive factors present in PhVa were comparable with those in other *Phaseolus vulgaris* varieties. However, the lectins of PhVa were predominantly of the leucoagglutinating type, and concentrated in the albumin protein fraction. Rats fed a diet (110 g total protein, 16·0 MJ/kg) in which PhVa meal provided about half of the protein excreted high levels of N in faeces and urine, and grew more slowly, than rats fed a high-quality control diet (*ad libitum* or pair-fed). Small intestine, large intestine and pancreas weights were increased (by almost 100 %, $P<0·05$), whilst skeletal muscle, thymus and spleen weights were reduced. Blood insulin (16·20 *v.* 0·50 mU/l, $P<0·05$, thyroxine, glucose, protein (60·5 *v.* 48·3 g/l, $P<0·05$) and LDL-cholesterol were lowered, whilst glucagon (155·3 *v.* 185·4 ng/l, $P<0·05$), triiodothyronine and urea were elevated, as were urinary urea, creatinine and glucose. These changes in the local (gut) and systemic metabolism of rats were probably mediated primarily by lectins in PhVa, which were concentrated in the albumin protein fraction, whereas in many other *Phaseolus vulgaris* lines they are distributed across the globulin and albumin fractions.

***Phaseolus vulgaris* L.: Blood composition: Urine composition: Hormones**

Legumes can be good dietary sources of protein and energy. Furthermore, they contain fibre, vitamins and bioactive compounds that may be useful in promoting health (Caroll & Kurokska, 1995; Messina, 1995; Persky & van Horn, 1995; Adlercreutz *et al.* 2000; Anthony, 2000). In particular, some legumes appear to have anticarcinogenic or hypolipidaemic properties that have been associated with the presence of bioactive factors, such as phytate, tannin, saponin, trypsin inhibitors and lectins in the seeds (Rao & Sung, 1995; Ali & Muzquiz, 1998; Grant, 1999*a*,*b*; Pryme *et al.* 1999). Globulin proteins and fibre may also have roles in the hypolipidaemic effects of legumes (Potter & Steinmetz, 1995; Ali & Muzquiz, 1998).

The bean (*Phaseolus vulgaris*) is extensively cultivated in western Europe because its nutritional value after heat-treatment is high (Grant, 1991). However, the nutritional quality of raw *Phaseolus vulgaris* is poor, primarily due to the constituent lectins (haemagglutinins). These are generally present in high concentrations and can be detrimental if consumed in quantity (Pusztai, 1991; Bardocz *et al.* 1996). However, the lectins may also have significant beneficial effects if they are present at low to moderate levels in the diet (Pusztai, 1991; Bardocz *et al.* 1996; Grant, 1999*b*). In addition, *Phaseolus vulgaris* contains a number of other bioactive substances with possible health-promoting properties (Ali & Muzquiz, 1998). European citizens have increased their consumption of beans due to nutritional claims that beans provide important nutrients and play an important role in preventing heart disease, diabetes and other disorders.

Phaseolus vulgaris varieties differ greatly in their composition and content of bioactive factors (Jaffé *et al.* 1973; Sgarbieri, 1989). Hence, the manner and extent to which they alter the metabolism of consuming animals is also

Abbreviation: PhVa, *Phaseolus vulgaris* L. var. *athropurpurea*.

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likely to vary. The present study attempts to show the nutritional and physiological effects on growing rats, and the toxic potential, of raw *Phaseolus vulgaris* L. var. *athropurpurea* (PhVa), frequently found in Europe.

Materials and methods

Legume analysis

Certified PhVa seeds were purchased from the Department of Agriculture of the Government of Navarra. Crude fibre, diethyl ether extract and N content were determined according to standard Association of Official Analytical Chemists (1990) methods. The amino acid composition of acid-digested samples of raw legume and PhVa-containing diet was determined using an HPLC system in combination with the Pico Tag method (Llames & Fontaine, 1994). Cystine and methionine were estimated from hydrolysates of performic acid-oxidised samples. Digestible and non-digestible carbohydrates were evaluated by the method of Rubio *et al.* (1991). Fatty acids in raw seeds were analysed by GC (Hendriksse & Harwood, 1986). Total phytate content was determined by ion-exchange chromatography (Frühbeck *et al.* 1995), and total tannins by the vanillin colorimetric procedure (Deshpande & Cheryan, 1987). Total saponin content was evaluated by TLC (Johnson *et al.* 1986). Albumin and globulin protein fractions were prepared as described previously (Carvalho, 1992; Bardocz *et al.* 1996), analysed by SDS-PAGE (Hajos *et al.* 1985), and lectins isolated and determined (Martinez-Aragón *et al.* 1995; Bardocz *et al.* 1996). Trypsin inhibitory activity was measured according to Armour *et al.* (1998).

Diets, animals and experimental design

Two isoproteic and isonergetic diets, containing about 110 g protein and 16·00 MJ metabolisable energy/kg, were formulated (Table 1). PhVa was included at 250 g DM/kg diet. In general, this is the maximum inclusion level with this type of seed meal that can be used without causing rapid weight loss and mortality in young rats (Grant *et al.* 1995). At this level of inclusion, PhVa provided about 55 g protein/kg diet. The protein content of the diet was made up to 110 g/kg by addition of lactalbumin. The control diet contained lactalbumin as the sole source of protein.

The rat study protocol was approved by the Committee of Animal Care of the Centro de Investigaciones en Farmacobiología Aplicada of the University of Navarra, and was carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

Thirty male Wistar albino rats (*Rattus novergicus*), bred in the University of Navarra Animal Unit and weighing about 60–70 g, were placed in individual metabolism cages (PANLAB, Barcelona, Spain) in a room with controlled temperature (20 ± 2°C), relative humidity (45 %), air laminar flow and light–dark cycle (light on from 08.30 to 20.30 hours). Water was freely available at all times. The animals were allowed to adapt to being in metabolism cages for 3 d, during which time they were given free access to the control diet (Table 1).

Table 1. Composition of experimental diets

	Diet	
	Control	PhVa
Ingredients (g/kg)		
Raw PhVa*	0·0	250·0
Lactalbumin*†	137·5	77·0
Sucrose	231·2	182·0
Maize starch	454·3	347·0
Olive oil	80·0	77·0
Mineral mix‡	35·0	35·0
Vitamin mix§	10·0	10·0
Cellulose	50·0	20·0
Choline‖	2·0	2·0
Calculated composition		
Total protein (g/kg)	108·0	110·0
Crude energy (MJ/kg)	17·0	16·8
Metabolisable energy (MJ/kg)	16·0	15·8

PhVa, *Phaseolus vulgaris* L. var. *arthropurpurea*.
* Amino acid profile of PhVa *v.* lactalbumin respectively (g/kg protein): aspartate 36·0 *v.* 122·1, threonine 36·0 *v.* 39·0, serine 15·0 *v.* 12·9, glutamic acid 43·0 *v.* 99·7, glycine 10·0 *v.* 5·8, alanine 11·0 *v.* 4·2, valine 43·0 *v.* 55·0, isoleucine 38·0 *v.* 49·0, leucine 62·0 *v.* 79·0, tyrosine 32·0 *v.* 41·0, phenylalanine 42·0 *v.* 51·0, lysine 50·0 *v.* 60·0, histidine 21·0 *v.* 25·0, arginine 44·0 *v.* 49·0, proline 1·0 *v.* 4·3, methionine plus cysteine 29·0 *v.* 44·0, tryptophan 9·0 *v.* 16·0.
† Lactalbumin (Sigma, St Louis, MO, USA) contained (g/kg): protein 800, lactose 40.
‡ AIN-76 (Dyets Inc., Bethlehem, PA, USA).
§ AIN-76 A (Dyets Inc., Bethlehem, PA, USA).
‖ Choline (Sigma) was mainly in the bitartrate form (990 g/kg).

Rats were then randomly assigned to three groups each of ten animals and fed test or control diet exclusively for 11 d. Group 1 was given free access to the control diet, group 2 had free access to the PhVa-containing diet and group 3 was pair-fed with the control diet in amounts equal to the daily intakes of rats given access to the PhVa-based diet *ad libitum*. Urine, in sulfosalicylic acid (120 g/l), and faeces were collected daily and immediately frozen at −20°C. Body weight, food and water intake were recorded daily.

At the end of the experiment, food was removed for 12 h. The animals were then lightly anaesthetised with diethyl ether and samples of blood collected from the retrorbitary plexus into chilled heparinised tubes. Blood samples were centrifuged (2000 ***g*** for 10 min) and the plasma frozen at −20°C. The animals were then killed by decapitation whilst still anaesthetised. The stomach, small intestine, caecum and colon were removed and the lumen contents flushed out. The pancreas, spleen, liver, kidneys, adrenals, thymus, lungs, heart, testes and gastrocnemius, soleus, extensor digitorum longus and tibialis digitorum longus muscles were also dissected out and weighed.

Analyses of faeces, plasma and urine

Total N content was determined by a micro-Kjeldhal method (Association of Official Analytical Chemists, 1990). Endogenous urinary N and faecal metabolic N were calculated by the procedure of Araya *et al.* (1974). Plasma glucose, urea, creatinine, ureate, triacylglycerol, total cholesterol, HDL-, VLDL- and LDL-cholesterol and

total protein and urinary glucose, urea, ureate and creatinine were determined by autoanalyser using kits (Hitachi 747; Boehringer Mannheim, Mannhein, Germany). Plasma albumin and globulin fractions were evaluated using an electrophoretic kit SPE Paragon (Beckman Instruments, Madrid, Spain) and commercial radioimmunoassay kits (CIS Biointernational, CA, USA) were used to determine insulin, corticosterone, glucagon, triiodothyronine, thyroxine and testosterone.

Statistical analysis

Statistical analysis was done by one-way ANOVA in combination with the Tukey test using the Instat Statistical Package (GraphPad Software Inc., San Diego, CA, USA). Differences were considered statistically different when $P \leq 0{\cdot}05$.

Results

Legume composition

PhVa contained a relatively high protein content of 220 g/kg, of which the extractable protein was predominantly of globulin type (Table 2). It also contained 560 g carbohydrate, 12 g lipid and 50 g fibre/kg DM. The amino acid profile of the protein was (g/kg): aspartic acid 66·0, threonine 24·5, serine 32·7, glutamic acid 94·9, glycine 22·0, alanine 23·2, valine 28·0, isoleucine 24·2, leucine 42·3, tyrosine 19·7, phenylalanine 32·0, lysine 48·9, histidine 16·4, arginine 37·3, proline 2·7, methionine plus cysteine 5·1, tryptophan 6·0. It was thus deficient in a number of essential amino acids when compared with a high-quality protein such as lactalbumin. The S amino acids (cystine and methionine) were limiting, followed by tryptophan. PhVa also had a high carbohydrate content, with starch and sucrose being the main constituents (Table 2).

Only moderate amounts of lipid were present in the seed meal (Table 2). However, these lipids were rich in linolenic acid, which comprised more than half of all the fatty acids, and in linoleic and palmitic acid.

The seed meal contained significant levels of bioactive factors (Table 2). Lectin, saponin and phytate levels were high. The lectins were found (by SDS-PAGE) to comprise two protein subunits, leucoagglutinating type and erythroagglutinating type, of >30 kDa; the leucoagglutinating-type was the predominant form present (Table 2). On fractionation, the lectins concentrated in the albumin fraction with no detectable lectin activity being found in the main protein (globulin) fraction.

Table 2. Composition (g/kg) of *Phaseolus vulgaris* var. *athropurpurea**

(Mean values with their standard errors for five samples)

Components	Mean	SEM
Ash	43·3	0·5
Fatty acids (g/kg total fat)		
Myristic	1·6	1·0
Palmitic	119·8	12·0
Stearic	13·5	0·2
Oleic	84·9	7·0
Linoleic	240·4	10·0
Linolenic	532·6	37·8
Other	7·2	0·1
Total protein (N × 5·4)	218·7	12·0
Albumin	41·0	2·5
Globulin†	125·2	4·0
Total carbohydrate	560·7	25·0
Starch	341·0	15·0
Sucrose	191·0	8·5
Raffinose	9·0	0·1
Stachyose	44·0	1·0
Verbascose	1·0	0·1
NSP constituents (g/kg NSP)		
Arabinose	36·0	1·0
Mannose	6·0	1·0
Galactose	13·0	0·1
Glucose	374·0	36·0
Inositol	4·0	1·0
Uronic acid	25·0	1·0
Ramnose	2·0	0·1
Fucose	2·0	0·1
Xylose	1·0	0·01
Antinutritional factors		
Phytates	11·7	0·8
Tannins (catechin equivalents)	6·5	0·7
Saponins	35·0	1·3
Trypsin inhibitors	4·7	0·3
Total lectins	18·0	1·9
Subunits (g/kg total lectins)		
Leucoagglutinins	678·0	7·0
Erythroagglutinins	322·0	7·0

* For details of analytical procedures, see p. 312.
† No detectable lectin.

Nutritional experiments

Rats fed a diet containing PhVa had reduced food intakes (Table 3). In addition, they grew very little over the 11 d experimental period (Fig. 1; Table 3). In contrast, pair-fed controls gained weight steadily, albeit at rates less than those of controls given unrestricted access to diet. The protein efficiency ratios obtained for PhVa were therefore low compared with those for control diet (fed *ad libitum* or pair-fed). This appeared to be a result of impaired net absorption of N and low retention of absorbed N (Table 3). The rats did not appear to adapt to the PhVa diet. Thus, the N utilisation, protein efficiency values and daily weight-gains achieved between days 8 and 11 were similar to those over the initial 7 d (Table 3).

Significant ($P<0{\cdot}05$) excretion of glucose in urine (552 (SE 45) mg/l) was evident with rats fed PhVa, whilst none was detected in the urine of either set of controls (Table 5). Urinary urea (control 1·25 (SE 0·01), PhVa-fed 2·25 (SE 0·01), pair-fed control 0·97 (SE 0·01) g/l) and creatinine (control 338 (SE 24), PhVa-fed 551 (SE 31), pair-fed control 386 (SE 31) mg/l) were also significantly increased by intake of PhVa diet. Uric acid was, however, unaffected (control 212 (SE 13), PhVa-fed 249 (SE 23), pair-fed control 386 (SE 31) mg/l).

The relative weights of several organs and tissues were significantly altered following consumption of PhVa by rats for 11 d (Table 4). Those of the small intestine and pancreas in particular were greatly increased compared with those of control animals (*ad libitum* or pair-fed).

Table 3. Intake, weight gain, protein utilisation and nitrogen balance for growing rats fed for 11 d on legume (*Phaseolus vulgaris* L. var. *athropurpurea*, L) or lactalbumin (control, C) diet *ad libitum* or pair-fed with control diet (CP) to match intakes of rats fed L*

(Mean values with their standard errors for ten rats per group)

	Diet					
	C		L		CP	
	Mean	SEM	Mean	SEM	Mean	SEM
Day 1–7:						
Food intake (g/kg BW per d)	156[a]	20	114[b]	10	105[b]	10
Protein intake (g/kg BW per d)	16[a]	1	10[b]	1	11[b]	1
Lectin intake (mg/kg BW per d)	–		513	45	–	
Weight gain (g/d)	5·9[a]	0·2	0·6[c]	0·1	1·9[b]	0·2
Protein efficiency ratio	2·7[a]	0·1	0·5[c]	0·1	1·7[b]	0·1
N retention (mg/kg BW per d)	1270[a]	90	230[c]	90	550[b]	80
True digestibility (%)	98·8[a]	1·0	61·6[b]	2·0	99·3[a]	2·0
True biological value (%)	89·8[a]	1·3	49·5[c]	3·2	80·1[b]	1·3
Net protein utilisation (%)	88·8[a]	1·3	31·4[c]	2·2	79·8[b]	1·2
Day 8–11:						
Food intake (g/kg BW per d)	146[a]	7	117[b]	4	108[c]	6
Protein intake (g/kg BW per d)	14[a]	1	11[b]	1	10[b]	1
Lectin intake (mg/kg BW per d)	–		527	18	–	
Weight gain (g/d)	6·8[a]	0·4	0·7[c]	0·1	2·5[b]	0·4
Protein efficiency ratio	4·4[a]	0·07	0·7[c]	0·01	2·3[b]	0·01
N retention (mg/kg BW per d)	1400[a]	100	270[c]	60	600[b]	70
True digestibility (%)	99·7[a]	1·4	62·8[c]	1·2	99·3[a]	1·2
True biological value (%)	91·8[a]	1·4	37·3[c]	2·7	86·1[b]	1·3
Net protein utilisation (%)	90·1[a]	1·3	23·3[c]	2·1	85·2[b]	1·5

BW, body weight.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 312.

Large intestine and lung weights were also slightly elevated whilst spleen, thymus and skeletal muscle weights were reduced. In contrast, liver, adrenal and heart weights appeared unaffected by intake of PhVa.

Hormone and blood analysis

Plasma insulin was greatly reduced as a result of intake of PhVa by rats (Table 5). Thyroxine was also reduced, whilst glucagon and triiodothyronine concentrations were slightly increased (Table 5). Corticosterone and testosterone also appeared to be altered. However, this may have been an effect of intake restriction, since the levels of these hormones were affected in a similar manner by restricting intake of the control diet (Table 5). In contrast, insulin, glucagon, triiodothyronine and thyroxine concentrations in controls were not significantly affected by intake restriction.

Plasma glucose levels were very low in rats that had been fed PhVa for 11 d (Table 5). Blood protein (albumin and α_1- and γ-globulin) was also decreased, as was LDL-cholesterol. In contrast, urea, ureate and HDL-cholesterol concentrations in the blood were increased (Table 5). Plasma triacylglycerol concentrations in rats fed PhVa were greater than those of pair-fed controls, but similar to those in rats given the control diet *ad libitum*. LDL-cholesterol was increased and VLDL-cholesterol decreased in rats given a restricted intake of the control diet.

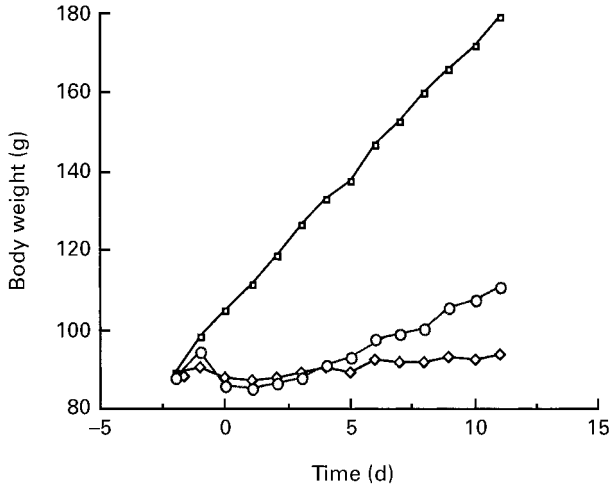

Fig. 1. Growth of Wistar rats fed control diet (C, □), *Phaseolus vulgaris* L. var. *arthropurpurea* diet *ad libitum* (L, ◇) or pair-fed with control diet (CP, ○). For details of diets and procedures, see Table 1 and p. 312. Values are means for ten rats per group.

Discussion

PhVa contained about 220 g protein/kg and the extractable proteins were predominantly globulin in nature, and cystine and methionine were the limiting amino acids. Its general composition was thus comparable with that of most other *Phaseolus vulgaris* varieties cultivated in Europe and South America (Pusztai *et al.* 1979; Sgarbieri, 1989; Codex Alimentarious Commission, 1990). Equally, the levels of bioactive factors, such as phytates, saponins,

Table 4. Organ and muscle weights in growing rats fed for 11 d on legume (*Phaseolus vulgaris* L. var. *athropurpurea*, L) or lactalbumin (control, C) diet *ad libitum* or pair-fed with control diet (CP) to match intakes of rats fed L*

(Mean values with their standard errors for ten rats per group)

	Diet					
	C		L		CP	
	Mean	SEM	Mean	SEM	Mean	SEM
Organs						
Stomach (g/kg BW)	5·8[b]	0·2	8·2[a]	0·6	7·2[a]	0·4
Small intestine (g/kg BW)	22·2[b]	0·9	52·7[a]	1·4	20·8[b]	0·8
Large intestine (g/kg BW)	8·3[b]	0·2	10·0[a]	0·4	7·2[c]	0·2
Pancreas (g/kg BW)	2·6[b]	0·1	4·8[a]	0·02	2·4[b]	0·2
Spleen (g/kg BW)	2·7[a]	0·1	2·0[b]	0·1	2·6[a]	0·1
Kidneys (g/kg BW)	3·9[b]	0·1	4·4[a]	0·1	4·3[a]	0·1
Thymus (g/kg BW)	2·8[b]	0·2	1·6[c]	0·1	3·9[a]	0·1
Lungs (g/kg BW)	4·1[b]	0·1	5·1[a]	0·2	4·2[b]	0·2
Testes (g/kg BW)	5·1[b]	0·2	7·9[a]	0·2	7·0[a]	0·3
Muscles						
Gastrocnemius (g/kg BW)	5·4[b]	0·1	4·3[c]	0·1	5·9[a]	0·1
Tibialis anterior (g/kg BW)	1·9[b]	0·1	1·5[c]	0·1	2·2[a]	0·1
Extensor digitorum longus (mg/kg BW)	401[b]	19	394[b]	9·10	490[a]	15
Soleus (mg/kg BW)	462[a]	1	430[b]	1	463[a]	1

BW, body weight.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 312.

trypsin inhibitors and lectins found in PhVa, were comparable with those in most other varieties (Pusztai *et al.* 1979; Sgarbieri, 1989; Codex Alimentarious Commission, 1990).

The bulk of the anti-nutritional effects associated with consumption of raw *Phaseolus vulgaris* seeds are associated with the constituent lectins (Pusztai, 1991; Grant, 1999*b*). The lectins of PhVa were structurally similar to those in the majority of other *Phaseolus vulgaris* lines (Pusztai *et al.* 1979; Sgarbieri, 1989) in that they comprised five tetrameric isolectins formed from combinations of two,

Table 5. Plasma variables of rats fed for 11 d on legume (*Phaseolus vulgaris* L. var. *athropurpurea*, L) or lactalbumin (control, C) diet *ad libitum* or pair-fed with control diet (CP) to match intakes of rats fed L*

(Mean values with their standard errors for ten rats per group)

	Diet					
	C		L		CP	
	Mean	SEM	Mean	SEM	Mean	SEM
Insulin (mU/l)	16·20[a]	0·50	0·50[b]	0·01	17·70[a]	1·33
Glucagon (ng/l)	155·30[b]	1·7	185·4[a]	7·7	134·8[c]	6·1
Triiodothyronine (nmol/l)	0·93[b]	0·04	0·88[b]	0·06	1·05[a]	0·03
Thyroxine (nmol/l)	51·8[a]	0·2	37·3[b]	1·7	51·3[a]	3·2
Corticosterone (U/l)	60[b]	5·2	163·3[a]	15·1	150·8[a]	50·0
Testosterone (ng/l)	280·0[a]	20	200·0[b]	10	200·0[b]	10
Glucose (mg/l)	966·7[b]	18·4	386·7[c]	37·7	1215[a]	38·6
Urea (mg/l)	232·5[b]	50·1	450·0[a]	30·1	152[b]	27·4
Ureate (mg/l)	16·8[a]	1·3	11·2[b]	0·5	18·3[a]	1·1
Creatinine (mg/l)	4·3[a]	0·3	4·0[a]	0·1	4·3[a]	0·3
Triacylglycerol (mg/l)	1095·0[a]	200	1038·0[a]	182	400·0[b]	56·9
Total cholesterol (mg/l)	856·7[a]	34·2	920·0[a]	27	870·0[a]	30·8
HDL-cholesterol (mg/l)	460·0[b]	7·0	582·5[a]	26·6	475·0[b]	5·0
VLDL-cholesterol (mg/l)	203·5[a]	25·9	207·5[a]	36·5	76·0[b]	9·0
LDL-cholesterol (mg/l)	193·2[b]	40·4	80·0[c]	15·5	369·0[a]	27·5
Total protein (g/l)	60·5[a]	1·2	48·3[c]	0·9	57·5[b]	0·7
Albumin (g/l)	48·6[a]	1·2	40·7[b]	1·3	46·5[a]	0·8
α_1-Globulin (g/l)	3·1[a]	0·4	2·2[b]	0·3	3·4[a]	0·2
α_2-Globulin (g/l)	1·9[a]	0·1	1·4[a]	0·3	1·7[a]	0·3
β-Globulin (g/l)	1·3[a]	0·1	1·0[a]	0·1	1·2[a]	0·2
γ-Globulin (g/l)	5·7[a]	0·3	3·3[c]	0·4	4·7[b]	0·4
Albumin: globulin ratio (g/g)	4·10[b]	0·3	5·38[a]	0·5	4·24[b]	0·1

[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 312.

the erythroagglutinating and leucoagglutinating subunits (Martinez-Aragón *et al.* 1995). However, the proportions of the subunits present differed greatly from most other lines. Leucoagglutinating type was predominant, with the isolectin L_4 accounting for over 70 % of the seed lectins and E_4 being present only in negligible amounts (Martinez-Aragón *et al.* 1995). In addition, the lectins of PhVa were concentrated in the albumin protein fraction, whereas in many other *Phaseolus vulgaris* lines they are distributed in the globulin and albumin fractions (Pusztai *et al.* 1979; Sgarbieri, 1989). Protein isolates or concentrates prepared from PhVa would thus be expected to contain little or no lectin, since the albumin fraction is removed during their preparation.

Relating to the nutritional performance, food intake by rats fed the diet containing PhVa was reduced. This may have been due to poor palatability, amino acid imbalances in the diet (cystine and methionine were present at approximately 64 % of requirement) or the action of bioactive factors (Pusztai *et al.* 1979; Sgarbieri, 1989). In particular, dietary lectins and trypsin inhibitors trigger release *in vivo* of hormones, such as cholecystokinin (Southon *et al.* 1987; Herzig *et al.* 1997; Grant *et al.* 1999, 2000), which greatly slow down stomach emptying and movement of digesta through the small intestine. Lectins also cause gut damage and promote bacterial overgrowth of the gut (Pusztai, 1991), which may have further reduced the voluntary food intake of the rats.

Weight gains by rats fed PhVa were only 28–35 % of those of pair-fed controls. This was a far greater reduction than could be explained by the reduced intake or amino acid deficiencies in the diet. Digestion and absorption of ingested N appeared to be low (about 38 % N was excreted in faeces), as was N retention (equivalent to 35–39 % of intake excreted in urine). The urinary N excretion (mainly urea) was at least 57–75 % greater than that which would be accounted for by amino acid (S amino acids) deficiencies in the diet. This suggests that the poor growth of rats fed PhVa was a result of impaired net absorption of N and low retention of absorbed N. The poor net absorption is likely to be a result of poor digestion of the dietary protein coupled with greatly increased outputs of endogenous N (mucus, sloughed-off epithelial cells, etc.) induced by PhVa (Pusztai *et al.* 1979; Fairweather-Tait *et al.* 1983; Pusztai, 1991). Impaired retention of N may have been due both to amino acid deficiencies in the diet and changes in systemic metabolism, elicited by dietary components, that prevented deposition of N (Grant, 1999*b*).

The weight of the small intestine increased approximately 2·5-fold as a result of consumption of PhVa. Similar changes were evident in rats fed lectin (520 mg/kg body weight per d) from *Phaseolus vulgaris* var. *processor* (Oliveira *et al.* 1988; Bardocz *et al.* 1995) and were likely to have been due to crypt cell hyperplasia, increased epithelial cell size, thickening of the smooth muscle layer and increased production of mucus (Pusztai, 1991; Bardocz *et al.* 1995). Rates of epithelial cell turnover would also have been greatly increased (Pusztai, 1991; Bardocz *et al.* 1995).

This PhVa-induced growth of the small intestine would have had major nutritional implications for the animals. The whole of the small intestine epithelium is normally replaced every 72 h. In addition, there is extensive synthesis and secretion of mucus to maintain a barrier layer on the gut surface. As a result, the normal healthy gut accounts for 20–35 % of whole-body protein turnover despite forming only 3–6 % of body weight (Reeds *et al.* 1999). However, turnover times can be reduced to 16–24 h and cell proliferation rates increased when the intestine is stimulated to grow by lectin (King *et al.* 1986; Pusztai, 1991; Bardocz *et al.* 1995, 1996). The already high requirements of the gut for nutrients are therefore greatly elevated. Indeed, at lectin intakes of about 520 mg/kg body weight per d, they may exceed nutrient intake (Pusztai, 1991). In this case, lectin-promoted gut growth appears to be preferentially supported from body reserves (Pusztai, 1991; Bardocz *et al.* 1992).

Pancreatic weights were almost doubled in rats fed PhVa for 11 d. This was probably due to the combined action of the lectins and trypsin inhibitors, both of which can trigger release of cholecystokinin from neuroendocrine cells in the intestine, thereby initiating hypersecretion of pancreatic digestive enzymes and subsequently promoting enlargement of the pancreas (Grant, 1999*a*,*b*; Grant *et al.* 1999). These prolonged effects on the pancreas also greatly increase its need for nutrients.

The total requirements for nutrients to support growth of the small intestine, pancreas and, to a lesser extent, the large intestine in rats fed PhVa (about 520 mg lectin/kg body weight per d) for 11 d may thus have been close to or exceeded intake. This may therefore have led to mobilisation of body reserves to meet the needs of the rapidly growing tissues (Pusztai, 1991; Grant 1999*b*). The low blood protein and glucose levels, high blood urea, elevated urinary urea and creatinine and the loss of skeletal muscle observed in the present study may thus reflect the occurrence of such catabolic changes in the body. The observed hormonal changes, particularly in insulin and glucagon, would facilitate the switch to net catabolism.

Fasted blood glucose levels of rats after 11 d on the PhVa diet were low. This was consistent with findings with other cultivars (Pusztai, 1991; Bardocz *et al.* 1996). Body reserves are generally low at this stage if rats have been fed with raw beans and blood glucose levels are maintained primarily from dietary sources. The levels therefore drop dramatically on removal of food (Carvalho, 1992; Bardocz *et al.* 1996). In addition, gluconeogenesis might contribute to glucose homeostasis in this situation, as well as in other studies (Raju *et al.* 2001).

Excretion of glucose in urine by PhVa-fed rats was high. This was consistent with the low insulin levels in these animals, but not with the view that net catabolism of reserves occurred to support tissue growth preferentially. Other mechanisms may therefore be involved. Lectins have been shown to inhibit insulin synthesis and secretion *in vivo*, either by direct action on the pancreas or indirectly through the release or regulatory hormones (Carvalho, 1992; Bardocz *et al.* 1996; Grant, 1999*b*). This may lead to rapid and excessive breakdown of body reserves. Since low insulin levels would also impair the efficacy with which the released components could be reutilised,

increased excretion in the urine is likely. This would suggest that the systemic metabolic changes observed in rats fed PhVa may be due to catabolism of body reserves specifically to support rapid lectin-mediated tissue growth and unregulated catabolism triggered by lectins, either directly or via hormone imbalances caused by their interactions with endocrine tissues (Pusztai, 1991; Grant, 1999*b*).

The weights of the thymus and spleen were reduced in PhVa-fed rats. This is consistent with reduced humoral and cellular immunocompetence noted in animals fed PhVa (Marzo *et al.* 1991) and may be due to the poor nutritional status of the animals. However, dietary lectins have been shown to reduce thymus weights in the absence of any nutritional impairment (Pusztai, 1991). The changes in the thymus and spleen may thus also be due to the action of the lectins in PhVa.

Consumption of raw PhVa reduced serum LDL-cholesterol and increased HDL-cholesterol in rats. This was particularly striking, since the rats used in the study were normo-cholesterolaemic. The mechanisms involved remain unclear, but may involve increased clearance of LDL-cholesterol and bile salt synthesis and excretion (Marzolo *et al.* 1993). The main bioactive factors, phytates, saponins and tannins, implicated in the hypocholesterolaemic effect of legumes are present in significant amounts in PhVa.

In conclusion, the present study, together with previous findings, suggests that changes in the local and systemic metabolism of rats were probably mediated primarily by lectins in PhVa, which are mainly located in the albumin protein fraction.

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