A survey of the nutritional and haemagglutination properties of legume seeds generally available in the UK

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1. Eighty-five samples from fifteen different legume seed lines generally available in the UK were examined by measurements of their net protein utilization by rats and by haemagglutination tests with erythrocytes from a number of different animal species. From these results the seeds were classified into four broad groups.

2. Group *a* seeds from most varieties of kidney (*Phaseolus vulgaris*), runner (*Phaseolus coccineus*) and tepary (*Phaseolus acutifolius*) beans showed high reactivity with all cell types and were also highly toxic.

3. Group *b*, which contained seeds from lima or butter beans (*Phaseolus lunatus*) and winged bean (*Psophocarpus tetragonolobus*), agglutinated only human and pronase-treated rat erythrocytes. These seeds did not support proper growth of the rats although the animals survived the 10 d experimental period.

4. Group *c* consisted of seeds from lentils (*Lens culinaris*), peas (*Pisum sativum*), chick-peas (*Cicer arietinum*), blackeyed peas (*Vigna sinensis*), pigeon peas (*Cajanus cajan*), mung beans (*Phaseolus aureus*), field or broad beans (*Vicia faba*) and aduki beans (Phaseolus angularis). These generally had low reactivity with all cells and were non-toxic.

5. Group *d*, represented by soya (*Glycine max*) and pinto (*Phaseolus vulgaris*) beans, generally had low reactivity with all cells but caused growth depression at certain dietary concentrations. This growth depression was probably mainly due to antinutritional factors other than lectins.

6. Lectins from group *a* seeds showed many structural and immunological similarities. However the subunit composition of the lectin from the tepary bean samples was different from that of the other bean lectins in this or any other groups.

The seeds of many edible legumes have long been known to contain proteins which agglutinate erythrocytes (Boyd, 1963). Some of these haemagglutinins (lectins) have been suggested to contribute to the poor nutritive quality of raw beans (Jaffe, 1969). Thus certain lines of kidney bean (*Phaseolus vulgaris*) are known to be toxic to human beings (Greibel, 1950; Noah *et al.* 1980), rats (Evans *et al.* 1974) and quail (*Coturnix coturnix japonica*) (Jayne-Williams & Burgess, 1974). The toxic factor in a sample of kidney beans has been shown to be identical with its constituent lectins and, consequently, the level of toxicity is directly related to the lectin content and hence haemagglutinating activity (Pusztai & Palmer, 1977).

Lectins from different seeds show an extent of specificity in their activity towards human and various animal erythrocytes (Landsteiner & Raubitschek, 1908). Thus a possible classification of the lectins present in different lines of kidney bean into four types has been suggested (Jaffe *et al.* 1972); of these only two types, namely those that exhibit activity towards trypsinated cow erythrocytes, are toxic (Jaffe, 1980).

However, it is not known how far these findings may be generalized. To find out whether the low nutritional performance of legume seeds can be directly attributed to their lectin content or if the haemagglutinating activity towards trypsinated cow erythrocytes can be used as an indicator of toxicity a general survey was undertaken of the agglutinin activity against a number of cells of different origin and the nutritional properties of untreated seeds commercially available in the UK.

Table 1. *Comparison of the net protein utilization (NPU) values of legume*

Group	Sample	Number of samples	Nitrogen (g/kg) Average	Nitrogen (g/kg) Range*	NPU: Diet containing 50 g sample-protein + 50 g casein/kg Average	NPU: Diet containing 50 g sample-protein + 50 g casein/kg Range	NPU: Diet containing 100 g sample-protein/kg Average	NPU: Diet containing 100 g sample-protein/kg Range
a	Runner bean (*Phaseolus coccineus*)	5	37·7	34·4–38·9	5	2–10	—	Negative Rats died‖
	Red or brown kidney bean (*Phaseolus vulgaris*)	10	40·3	36·0–44·0	—	Negative‡–14	—	Negative Rats died
	White kidney bean (*Phaseolus vulgaris*)	4	42·4	39·8–44·0	17	4–36	—	Negative Rats died
	Black kidney bean (*Phaseolus vulgaris*)	5	42·2	40·9–43·9	12	10–16	—	Negative Rats died
	Tepary bean (*Phaseolus acutifolius*)	4	51·3	50·0–52·8	10	8–13	—	Rats died Rats died
b	Winged bean (*Psophocarpus tetragonolobus*)	2	67·4	66·0–68·8	25	24–26	—	Negative Negative
	Baby lima bean (*Phaseolus lunatus*)	1	39·2		27		Negative	
c	Lentils (*Lens esculentus*)	7	47·5	44·3–49·6	53	49–55	44	43–46
	Peas (*Pisum sativum*)	5	45·5	40·9–49·1	60	54–66	52	43–62
	Chick peas (*Cicer arietinum*)	7	38·7	38·1–39·3	66	66–66	56	53–58
	Blackeyed peas (*Vigna sinensis*)	7	42·3	42·2–42·4	59	55–62	37	37–37
	Pigeon peas (*Cajanus cajan*)	1	34·9		60		50	
	Mung beans (*Phaseolus aureus*)	6	4·04	37·9–42·4	69	68–69	44	44–44
	Black mung beans (*Phaseolus aureus*)	1	38·5		60		35	
	Field or broad beans (*Vicia faba*)	9	48·7	41·0–55·2	59	52–68	48	39–57
	Aduki beans (*Phaseolus angularis*)	1	41·9		53		39	
d	Soya beans (*Glycine max*)	6	67·5	64·8–70·1	56	53–58	24	19–28
	Pinto beans (*Phaseolus vulgaris*)	4	40·6	39·1–42·1	50	45–55	10	9–11

Results for multiple samples are expressed as averages (arithmetic average) except for the haemagglutination activities where values were approximated to the nearest actual dilution value.

* The range is expressed in terms of the minimum and maximum values obtained.

MATERIALS AND METHODS

Seed samples

Samples of lentils (*Lens culinaris*), peas (*Pisum sativum*), chickpeas (*Cicer arietinum*), blackeyed pea (*Vigna sinensis*), pigeon pea (*Cajanus cajan*), mung bean (*Phaseolus aureus*), field, broad, ful or tic beans (*Vicia faba*), soya bean (*Glycine max*), aduki bean (*Phaseolus*

seeds and their haemagglutination activity towards various erythrocytes

Haemagglutination activity									
Rabbit blood cells		Pronase-treated rat blood cells		Trypsin–treated cow blood cells		Human blood cells			
						0+		AB+	
Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
6	1·5–12	1·5	0·8–1·5	6	3–12	195	98–390	195	98–390
†	6–98	1·5	0·4–6	†	1·5–98	195	98–390	195	98–390
12	6–24	6	1·5–12	†	3–780	195	98–390	†	12–390
12	6–24	3	1·5–6	†	1·5–49	†	24–390	†	24–390
6	1·5–12	1·5	0·8–1·5	195	49–390	98	24–195	24	24–24
12500	12500–12500	24	24–24	12500	12500–12500	390	195–780	195	195–195
12500		6		12500		12500		98	
†	49–780	24	6–49	12500	12500–12500	3120	780–3120	3120	1560–6250
98	49–195	24	12–49	12500	12500–12500	3120	3120–3120	3120	3120–3120
12500	12500–12500	†	98–1560	12500	6250–12500	12500	12500–12500	12500	12500–12500
12500	12500–12500	†	98–1560	12500	12500–12500	12500	12500–12500	12500	12500–12500
12500		98		12500		12500		12500	
12500	12500–12500	1560	390–3120	12500	12500–12500	12500	12500–12500	12500	12500–12500
12500		24		12500		12500		12500	
†	49–3120	†	24–780	12500	3120–12500	6250	3120–12500	6250	3120–12500
12500		24		3120			12500	12500	
†	24–390	390	195–390	3120	3120–3120	12500	12500–12500	12500	12500–12500
12500	6250–12500	98	49–98	6250	3120–6250	12500	12500–12500	12500	12500–12500

† Where the range of the haemagglutinin was much greater than the experimental error only minimum and maximum values obtained from different samples are given.

‡ NPU value was less than 0.

‖ The experiment had to be terminated within the 10 d period because the rats died.

angularis), runner bean (*Phaseolus coccineus*), lima or butter bean (*Phaseolus lunatus*), zebra bean (*Phaseolus zebra*) and of various kidney beans (*Phaseolus vulgaris*) were obtained from Aberdeen Grain and Herb Store (Aberdeen), Nature's Larder (Aberdeen), Real Foods (Edinburgh) and Fine Fare (Aberdeen). Black mung beans were obtained from Abrosia (Aberdeen). The winged bean (*Psophocarpus tetragonolobus*) and tepary bean (*Phaseolus*

acutifolius) samples were provided by the Mayaguez Institute of Tropical Agriculture (Puerto Rico), the *Phaseolus coccineus*, *Phaseolus leucanthius* and *Phaseolus lunatus* samples by the National Vegetable Research Station (Wellesbourne, Warwick) and the sultani bean and various broad bean samples by the Rowett Research Institute (Aberdeen). Lactic-unextracted casein was supplied by Glaxo Laboratories Ltd (Greenford, Middlesex).

Diets

Diets were prepared as described previously (Palmer *et al.* 1973) and contained a total of 100 g protein as casein or uncooked seed protein/kg or a combination of casein plus seed protein each at 50 g/kg. The protein sources were given without supplementation with any individual amino acids.

Haemagglutination assay

Blood samples were collected into preheparinized tubes and after collection were diluted twentyfold with saline (9 g sodium chloride/l). Rat erythrocytes were pretreated with pronase (0·2 mg/10 ml diluted erythrocytes for 30 min at 25°) and cow erythrocytes with trypsin (0·1 mg/10 ml diluted erythrocytes for 60 min at 25°) (Jaffe *et al.* 1972). Samples were visually sorted to ensure purity and then ground in a Wiley Laboratory Mill fitted with a 425 μm mesh grid. Flours were extracted with 0·04 M-sodium borate buffer, pH 8·0, for 16 h at 1° (flour-buffer 1:20, w/v). After centrifugation (78000 ***g*** for 60 min), the clear supernatant fractions were tested for haemagglutinin activity. The supernatant fractions were serially diluted using a Titertek medimixer (Flow Laboratories Ltd, Irvine) and mixed with an equal volume of diluted erythrocytes (final volume 0·1 ml). This gave the following sample concentrations (μg/ml) in the haemagglutinin assay:

Tube no....	1	2	3	4	5	6	7	8	9
	12500	6250	3120	1560	780	390	195	98	49
Tube no....	10	11	12	13	14	15	16	17	18
	24	12	6	3	1·5	0·8	0·4	0·2	0·1

The mixed samples were left for 16 h and then the amount of clumping was assessed by microscope. *Phaseolus vulgaris* var. Processor and var. Pinto III extracts (Pusztai *et al.* 1979) were included in each assay as standard controls. One unit of haemagglutination activity (H.U.) was defined as the amount of material per ml in the last dilution giving 50% agglutination. For comparison purposes, the activity of the various samples was given as the amount of material (μg) containing 1 H.U. The limits of experimental accuracy for this technique are ± 1 dilution and therefore samples of low or moderate haemagglutinin activity will have an apparently-wide range of error. For example, a sample with a titre of 6250 will have a range of accuracy of 12500–3120. The results for multiple samples were combined to produce an arithmetic average but because of the experimental error and, for comparison purposes, any value which was not equivalent to one of the sample concentrations used, was rounded up to the nearest actual value. For example, the average of 12500 and 6250 was given as 12500.

Nutritional evaluation

Net protein utilization (NPU) was determined by a method (Palmer *et al.* 1973) based on that described by Miller & Bender (1955).

Chemical analyses

Diets, carcass and ground faeces samples were analysed for moisture content and total nitrogen (Davidson *et al.* 1970). Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis was carried out on slabs of 17·6% polyacrylamide separation gel and 4%

Table 2. *Comparison of the net protein utilization (NPU) values and haemagglutinating activity towards various erythrocytes of the lima or butter bean* (Phaseolus lunatus) *and runner bean* (Phaseolus coccineus) *samples*

Sample	Nitrogen (g/kg)	NPU		Haemagglutination activity				
		Diet containing 50 g sample-protein + 50 g casein/kg	Diet containing 100 g sample-protein/kg	Rabbit blood cells	Pronase-treated rat blood cells	Trypsin-treated cow blood cells	Human blood cells	
							0^+	AB^+
Runner bean	40·4	ND	ND	49	3	49	195	195
Lima beans: 1	34·1	17	Rats died†	49	0·8	6	195	390
2	31·0	3	Rats died	3	1·5	1·5	49	98
3	31·0	47	15	12500	24	6250	12500	1560
Baby lima beans	39·2	27	Negative*	12500	6	12500	12500	98
Butter beans: 1	35·8	42	Negative	12500	49	12500	12500	98
2	34·9	44	9	12500	1·5	3120	12500	780
Lima bean	37·3	ND	ND	12500	12	6250	12500	780

ND, not determined.
* NPU value was less than 0.
† The experiment had to be terminated within the 10d experimental period because the rats died.

stacking gel (Laemmli, 1970) run on an LKB Multiphor apparatus. The samples were extracted with SDS (20 g/l), 2-mercaptoethanol (10 ml/l) to give a nominal concentration of 2·5–3·0 g protein/l. After heating at 100° for 15 min, 10 μl of each sample was applied to the polyacrylamide gel and run for 4 h at 2·1 mA/well. The gels were stained with a Coomassie Blue solution (5 g/l) and destained with a solvent mixture of methanol–acetic acid–water (8:1:8, by vol.).

RESULTS

On the basis of nutritional value and behaviour towards lectins (Table 1), the seeds fell into four broad groups.

Group *a*, which comprised red or brown, black and white kidney beans, runner beans and tepary beans, gave low NPU values (0–17) with mixed diets containing 50 g seed protein and 50 g casein/kg; furthermore, with diets containing 100 g seed protein/kg, negative NPU values were obtained or, in most cases, the rats died within the 10 d experimental period. These samples were therefore classed as highly toxic.

Group *b*, comprising winged beans and lima or butter beans, depressed growth with both diets. In mixed diets containing 50 g seed protein and 50 g casein/kg, NPU values of 25–27 were obtained whilst at 100 g seed protein/kg the corresponding NPU varied between negative and slightly positive values. In all cases, the rats survived the 10 d experimental period.

Group *c*, including lentils, peas, chick-peas, blackeyed peas, pigeon peas, mung beans, black mung beans, field or broad beans and aduki beans, gave good NPU values with both diets. With diets containing 50 g seed protein plus 50 g casein/kg average NPU values of 50–70 were obtained. At 100 g seed protein/kg the corresponding values were 35–56. These samples were therefore considered to be essentially non-toxic.

Group *d*, represented by soya and pinto beans, gave positive NPU values at both 50 and 100 g/kg, but whilst at 50 g seed protein/kg average NPU values of 50–56 were obtained, those at 100 g seed protein/kg were considerably lower at 24 (soya bean) and 10 (pinto bean).

Subdivision on the basis of nutritional performance correlated well with the erythrocyte specificities (Landsteiner & Raubitschek, 1908) of the seed constituent lectins. Group *a* samples had high lectin activities and agglutinated all the erythrocytes tested, group *b* agglutinated pronase-treated rat erythrocytes and human O^+ or AB^+ erythrocytes or both and group *c* agglutinated only rabbit or pronase-treated rat erythrocytes or both.

The results of the nutritional evaluation and haemagglutinating activity measurements obtained with various lima and butter bean samples were not clear (Table 2). Although the lima bean (*Phaseolus lunatus*) lectin is known to be blood-group specific for human A^+ erythrocytes (Jaffe, 1980), only four of the lima and butter bean samples of the six tested showed this type of specificity. These samples also gave intermediate NPU values indicating they were group *b* seeds. However, the other two lima bean samples reacted with all the erythrocytes tested, gave very low NPU values with 50 g/kg diets and with 100 g/kg diets the rats died during the experiment. This indicated they were group *a* seeds. Indeed, it was shown by SDS-polyacrylamide gel electrophoresis (Plate 1) that the subunit composition of these two lima bean samples was more similar to the more toxic runner bean than to that of genuine lima bean samples.

These results may have been due to incorrect labelling; other examples of incorrect labelling of seeds were also encountered. For example, some samples of pinto beans were found to be similar to the more toxic rose-cocoa (crab-eye) beans and a sample, sold as white kidney bean, was found to contain at least three different bean types.

DISCUSSION

Legume seeds could be separated into four broad groups. Within these groups the nutritional performance or the extent of toxicity of the seeds could be related to the general reactivity and the lectin content of the seed.

With group *a* and group *b* samples there was a good correlation between lectin content, specificity and NPU values; thus it was considered that group *a* samples exhibited a high extent of lectin-related toxicity and group *b* samples an intermediate extent of lectin-related toxicity. With group *c* samples there was no such correlation and these were considered to show no appreciable lectin-related toxic effects.

Although no single erythrocyte type could be used as an indicator, the potential lectin-related toxicity of seed samples could be predicted by measuring the haemagglutinating activity towards various erythrocytes. Thus, since group *d* samples failed to give a clear correlation between lectin content and NPU values and they agglutinated only rabbit or pronase-treated rat erythrocytes or both, it is suggested that other antinutritional factors might be mainly responsible for the growth depression found with diets containing these samples at 100 g seed-protein/kg.

With several of the seeds examined such as chick-peas, blackeyed peas, field or broad beans, soya beans and mung beans, the range of haemagglutinin activities found for individual batches of the seeds was somewhat greater than the experimental error. Since the values obtained for individual batches were reproducible, within experimental error, it was considered that these differences were a true reflection of the variability between commercial batches available in the UK.

The possibility of contamination of the commercial samples either by other seed species or by chemical and biological materials cannot be completely excluded.

It has previously been noted that of the group *a* samples the kidney (*Phaseolous vulgaris*) and runner (*Phaseolus coccineus*) beans are immunologically closely related, although not identical (Pusztai *et al.* 1983). However, the tepary bean (*Phaseolus acutifolius*) is the most distantly related of the group *a* beans (Pusztai *et al.* 1983). This was further supported by

the results obtained by SDS-polyacrylamide gel electrophoresis (Plate 1). Whilst kidney, runner and zebra beans gave patterns indicating molecular weights of approximately 30000 for the lectin subunit bands, no major bands were seen in corresponding positions with the tepary bean samples, thus indicating that the tepary bean lectin might be somewhat different from the other lectins within the group. This finding also suggested that similar nutritional effects or haemagglutinating reactivity of the different bean samples did not necessarily imply similarity in the identity of composition or structure of their constituent lectins.

The differences found with lima bean samples highlight the problem that many beans have similar markings and are of similar colour, size and shape and it cannot be guaranteed that seeds generally available will always be labelled with their correct botanical name. Therefore, whilst it is clear that a number of legume seeds are non-toxic, in practice all beans should be treated as potentially toxic, because of the problem of identification. Thus seeds should be fully hydrated and heated at 100° for a minimum of 10 min before use (Grant *et al.* 1982).

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EXPLANATION OF PLATE

Plate 1. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis patterns of various bean samples. (*a*) Lane 1, kidney bean (*Phaseolus vulgaris* var. Processor)+cytochrome *c*; lane 2, runner bean (*Phaseolus coccineus*); lane 3, lima-bean sample no. 1; lane 4, runner bean. (*b*) Lane 1, kidney bean+cytochrome *c*; lane 2, lima-bean sample no. 1; lane 3, baby lima bean; lane 4, butter-bean sample no. 1; lane 5, sultani bean; lane 6, lima or butter bean (*Phaseolus lunatus*); lane 7, *Phaseolus leucanthius*; lane 8, lima-bean sample no. 3; lane 9, lima-bean sample no. 2; lane 10, butter-bean sample no 2; lane 11, zebra bean (*Phaseolus zebra*); lane 12, pinto bean (*Phaseolus vulgaris*); lanes 13–19, tepary bean (*Phaseolus acutifolius*); lane 20, kidney bean+cytochrome *c*.