Nutritional Evaluation of RAZ-2, a New *Phaseolus vulgaris* Bean Cultivar Containing High Levels of the Natural Insecticidal Protein Arcelin 1[†]

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The nutritional properties of RAZ-2, a recently developed bean (*Phaseolus vulgaris*) line which, because of its high levels of the natural insecticidal protein arcelin 1 and low content of toxic lectin, PHA, is highly resistant to attack by bean weevils, have been studied with rats. Raw RAZ-2 bean was nontoxic but, as its proteins were less digestible and less well utilized than those of high-quality animal proteins and because of its stimulation of the growth of the gut and pancreas and increased lipolysis, growth of the rats was somewhat retarded. However, although the nutritional value of raw RAZ-2 bean was slightly below that expected from its chemical composition and low PHA content, the antinutritional effects of this line are less than those with most bean varieties. The toxicity of arcelin 1 is thought to be mainly directed against insects, but it is possible that arcelins may also contribute to the reduced but slightly deleterious effects of these raw beans. However, similar to the improvement of nutritional value in other bean cultivars, most of the antinutritive properties of RAZ-2 could be abolished by heating the fully hydrated beans at 100 °C for 10 min.

INTRODUCTION

Insect predation on crops is usually controlled by spraying with potent insecticides. However, insects quickly develop resistance to these chemicals. Moreover, potentially toxic residues from the insecticide can reach the animal and human food chain. It is, therefore, necessary to develop alternative means of controlling crop infestation by insects.

A number of wild strains of beans (*Phaseolus vulgaris*) from Mexico have been found to be highly resistant to bean weevils (*Acanthoscelides obtectus* and *Zabrotes subfaciatus*) (Cardona et al., 1990). Apparently, this is due to the presence of high levels of a novel protein, arcelin, in these seeds (Osborn et al., 1988a; Cardona et al., 1989, 1990; Minney et al., 1990).

The five arcelin variants, arcelins 1–5, that occur naturally have considerable structural and immunogenic similarities to kidney bean (*P. vulgaris*) lectins, PHA (Osborn et al., 1986, 1988a,b; Lioi and Bollini, 1989). However, unlike PHA, arcelins do not agglutinate native erythrocytes or bind to fetuin or thyroglobulin (Osborn et al., 1986, 1988a,b; Chrispeels and Raikhel, 1991).

Arcelins have been successfully transferred into cultivated bean lines by backcrossing and other breeding methods, and new insect-resistant cultivars have been developed (Harmsen et al., 1988; Cardona et al., 1990). Although it has been established that arcelins are highly deleterious to insects, nothing is known about their potential effects on domestic animals or humans. In this study, the nutritional properties of RAZ-2, a new bean line which contains high levels of arcelin 1 but a low concentration of PHA, have been evaluated in rats and compared with those of cultivar EMP-175, the insect-susceptible parent line of moderate PHA content and var. Processor (PROC), a toxic kidney bean cultivar of high PHA content. As there is increasing evidence that because of the lack of firewood in some developing countries, e.g. in Africa, or limited access to heat energy for a variety of reasons, people are forced to eat raw or not properly cooked nutritionally toxic beans, this study placed a heavy emphasis on nutritional testing of beans in the raw state.

MATERIALS AND METHODS

Seeds. *P. vulgaris* bean cultivars RAZ-2 (weevil resistant) and EMP-175 (susceptible parent) were supplied by the Centro International de Agricultura Tropical (Cali, Colombia), and var. Processor bean was purchased from Booker Seeds (Sleaford, Essex, U.K.). RAZ-2 bean is a double-inbred backcrossed line obtained by selection for the presence of arcelin 1 in early segregating populations and selection for insect damage levels in F3 and subsequent generations (Cardona et al., 1992). Seeds were ground in a Christy and Norris laboratory mill fitted with a 1 mm pore diameter grid. Boiled bean samples were prepared by heating fully hydrated seeds in distilled water (3:10 w/v) at 100 °C for 10 min (Grant et al., 1982), freeze-dried, and ground with a Moulinex coffee grinder.

Diets. Isocaloric (100 g of protein/kg) diets were prepared (Table I) as before (Grant et al., 1986). Protein was provided exclusively either by lactalbumin (control) or bean meal or by a

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Table I. Composition of Diets (Grams per Kilogram)

	diet						
	1	2	3	4	5	6	7
maize starch	500	374	176	54	92	104	142
potato starch	100	100	100	100	100	100	100
glucose	150	150	150	150	150	150	150
corn oil	150	150	150	150	150	150	150
minerals[a]	50	50	50	50	50	50	50
vitamins[a]	50	50	50	50	50	50	50
lactalbumin		126	64				
RAZ-2 bean				446			
EMP-175 bean					408		
Processor bean			260				
boiled RAZ-2						396	
boiled EMP-175							358
L-tryptophan			0.2	0.6	0.6	0.6	0.6
L-methionine			1.2	2.0	1.9	2.0	1.9
L-leucine				1.3	1.0	1.3	1.0
L-isoleucine				1.0	1.4	1.0	1.4
L-valine				0.5	1.3	0.5	1.3
silicic acid	0.4	0.4	0.4	0.4	0.4	0.4	0.4

[a] Vitamin and mineral mixtures formulated as per Grant et al. (1986).

1:1 (w/w) combination of bean protein and lactalbumin. Some of the diets were supplemented with individual amino acids to bring their levels up to the target requirements for rats (Coates et al., 1969). A protein-free diet was used as a negative control in some experiments.

Animals. Male Hooded-Lister rats (Rowett colony), reared and housed under standard conditions in the Small Animal Unit (specific pathogen-free) of the Rowett Research Institute, were weaned at 19 days and kept on a standard stock diet (Special Diet Services, Manea, Cambridgeshire, U.K.) in individual cages for 10 days. They were then prefed ad libitum on lactalbumin control diet (diet 2) for 3–4 days before the start of the experiment. Water was available at all times ad libitum.

Nutritional Studies ad Libitum. Net protein utilization (NPU), dry matter and nitrogen digestibility, and biological value (BV) were determined as before (Palmer et al., 1973). Rats were sorted into groups of four whose combined weights were similar to within 5 g. Each group was subsequently fed ad libitum on the appropriate test or control diets for 10 days. Fecal samples were collected daily between days 4 and 10. At the end of the experiment, rats were killed by ether anaesthesia overdose. The carcasses and fecal samples were freeze-dried and ground in a Moulinex coffee grinder.

Restricted Intake Experiments. Rats whose weights were between 80 and 82 g were selected, housed individually, and fed the appropriate test or control diet of 6 g/day per rat, given as two feeds at 9 a.m. and 5 p.m. for 10 days. Although this amount of food was below the daily voluntary intake, it was above that necessary to meet the minimum requirements for energy, minerals, and vitamins, which is estimated to be about 5 g of diet/day per rat for this size of rat. At the end of the experiment, rats were killed by ether anaesthesia overdose and dissected. Stomach, small intestine, caecum, and colon were removed and their contents washed out with ice-cold distilled water. Liver, kidneys, pancreas, spleen, thymus, lungs, heart, and soleus, plantaris, and gastrocnemius muscles from both hind legs were also taken and, together with the remainder of the carcass, freeze-dried and weighed.

Analytical Methods. Total N was estimated by an automated macro-Kjeldhal method (Davidson et al., 1970) and lipid content by extraction with 2:1 (v/v) chloroform/methanol as described earlier (Grant et al., 1986). Amino acid analysis was done as published previously (Grant et al., 1986). Hemagglutinating activity against native rabbit blood cells was estimated by a serial dilution procedure (Grant et al., 1983). The amount of material (micrograms of DM) which caused agglutination of 50% of the erythrocytes was defined as that containing 1 hemagglutinating unit (HU). For comparison, values were expressed as HU/mg DM.

Statistical analysis was by one-way analysis of variance using the Minitab computer program (Scottish Agricultural Statistics Service, Edinburgh, U.K.). When P values were <0.05, the significance of difference between groups was estimated by Student's t-test.

Table II. Composition of Bean (*P. vulgaris*) Cultivars RAZ-2 (Weevil Resistant) and EMP-175 (Weevil Susceptible)

	RAZ-2	EMP-175	
N, g/100 g of DM[a]	3.75	3.90	target[b]
Arg, g/16 g of N	6.87	6.57	5.0
His	3.38	3.08	2.5
Lys	6.88	6.48	6.0
Tyr	3.94	3.86	4.0
Try	ND[c]	ND	1.5
Phe	6.05	5.30	5.0
Met	1.25	1.68	4.5[d]
Cys	1.20	1.19	
Thr	5.67	4.70	4.0
Leu	6.67	7.11	8.0
Ile	3.94	3.61	5.0
Val	5.17	4.24	5.5
Asp	12.36	12.27	
Ser	7.01	6.57	
Glu	12.61	14.48	
Gly	3.95	3.57	
Ala	4.59	3.90	
Pro	3.16	3.50	
HU[e]/mg of DM	3	21	

[a] Dry matter. [b] Profile of essential and semiessential amino acids required by rats (Coates et al., 1969). [c] Not determined. [d] Combined requirement for cysteine and methionine. [e] Hemagglutinating units. For comparison, kidney bean var. Processor contained 40 HU/mg of DM.

RESULTS

The nitrogen contents of RAZ-2, and its susceptible parent, EMP-175, were similar, indicating that the protein (N × 6.25) content of these seeds was between 23.4 and 24.3% (Table II). Although the absolute concentrations of some individual amino acids varied slightly, the overall amino acid profiles of the seeds were quite close. In both beans, the contents of sulfur amino acids were low, providing only 54–64% of requirement for rats.

Inclusion of raw RAZ-2 bean in the diet as the sole source of dietary protein considerably depressed the food intake of rats compared with that of rats given lactalbumin control diet (Table III). Both protein and dry matter digestibilities were low and independent of food intake (Tables III and IV). However, lipids of both raw and boiled RAZ-2 diets

Table III. Nutritional Performance of Rats Fed ad Libitum with Diets (100 g of Protein/kg) Containing Raw or Heat-Treated Meal from the Bean Cultivars RAZ-2 (RAZ) or EMP-175 (EMP)

	diet					
	raw bean protein		boiled bean protein		2, lactalbumin control protein	1, protein-free control
	4, RAZ	5, EMP	6, RAZ	7, EMP		
weight change,[a] g	+34	−72	+191	+217	+233	−73
food intake[a]						
DM, g	348	237	531	571	537	243
N, g	6.16	3.98	8.91	9.69	9.48	0.27
digestibility						
DM, %	84	79	87	88	96	95
N, %	72	45	89	92	100	
food conversion						
gain/intake, g/g	0.10	neg[b]	0.36	0.38	0.43	
NPU, %	60	28	77	85	98	
BV, %	83	62	86	92	98	

[a] Per four rats over 10 days. Initial weights were 340–345 g/group of four rats. [b] Food conversion values are negative.

Table IV. Nutritional Performance of Rats Fed on Diets (6 g/Day per Rat) Containing Bean Meals or Lactalbumin (Control) for 10 Days[a]

	diet				
	4, raw bean RAZ	5, raw bean EMP	3, raw bean PROC + LA	6, boiled bean RAZ	2, control lactalbumin
weight change,[b] g	-1.7 ± 2.0^{a}	-14.6 ± 1.3^{b}	-11.1 ± 3.0^{b}	$+4.8 \pm 2.1^{c}$	$+10.9 \pm 2.2^{d}$
intake					
DM, g	60	60	60	60	60
N, mg	1023	974	991	981	1033
lipid, g	9.0	9.0	9.0	9.0	9.0
digestibility					
DM, %	86 ± 2^{a}	84 ± 2^{a}	83 ± 1^{a}	85 ± 2^{a}	96 ± 1^{b}
N, %	72 ± 2^{a}	49 ± 3^{b}	60 ± 4^{b}	89 ± 3^{c}	96 ± 2^{d}
lipid, %	91 ± 2^{a}	85 ± 1^{b}	84 ± 1^{b}	89 ± 1^{a}	93 ± 1^{a}
dried carcass					
DM, g	21.5 ± 0.7^{a}	18.3 ± 0.6^{b}	18.3 ± 1.1^{b}	25.0 ± 1.6^{c}	26.2 ± 1.3^{c}
N, g	2.4 ± 0.1^{a}	2.2 ± 0.1^{a}	2.2 ± 0.1^{a}	2.8 ± 0.1^{b}	2.8 ± 0.1^{b}
lipid, g	2.7 ± 0.4^{a}	1.8 ± 0.3^{b}	2.0 ± 0.2^{b}	4.7 ± 0.7^{c}	4.5 ± 0.7^{c}
utilization					
NPU	46 ± 6^{a}	28 ± 7^{b}	27 ± 7^{b}	76 ± 4^{c}	91 ± 5^{d}
BV	64 ± 6^{a}	57 ± 8^{b}	46 ± 7^{b}	86 ± 2^{c}	94 ± 3^{d}

[a] Values in a row with distinct superscripts are significantly different ($P < 0.05$). [b] Initial weights were 80–82 g.

were digested as efficiently as the lactalbumin control diet. Nevertheless, the body lipid content of rats after feeding the diet of raw RAZ-2 bean for 10 days was significantly less than that of the lactalbumin control. With cooked beans the body's lipid content increased to that of the lactalbumin group (Table IV). Overall, rats fed restricted amounts of raw RAZ-2 diet could only maintain their initial weight. Even when fed ad libitum, their growth was below that of the control rats fed either lactalbumin or cooked bean diets (Tables III and IV).

Diets containing raw EMP-175 as the only protein source were clearly more deleterious than those with the insect-resistant new cultivar. Rats lost weight rapidly on this diet, irrespective of whether they were fed restrictedly or ad libitum. Digestibility of both nitrogen and dry matter was low, food conversion ratio was negative, and net protein utilization was close to zero with EMP-175 fed ad libitum (Table III). Similar poor performance was found with rats fed restrictedly on this diet (Table IV).

Raw PROC beans were even more toxic and could not be tested without diluting the protein content of the diet by an equal amount of lactalbumin (Tables III and IV). Even then, rats lost weight rapidly during the 10-day experimental period.

The hemagglutinating activities of the beans differed considerably (Table II). However, the RAZ-2 bean had a low toxic PHA content. Thus, calculated from its red cell agglutination titer, the PHA content in RAZ-2 bean was only about 14% of that in EMP-175 and about 7% of that in the highly toxic PROC bean. Accordingly, the daily intakes of PHA by rats fed on raw EMP-175 or PROC (diluted with lactalbumin) diets were comparable: 51 000 HU/day on EMP-175 and 62 000 HU/day on PROC. This was matched by their essentially similar nutritional value. In contrast, the daily PHA intake of rats given raw RAZ-2 bean was only 8000 HU/day, with a comparable increase in the nutritional value of this diet.

The weights of small intestine, caecum, and colon of rats fed raw RAZ-2 bean were increased in comparison with those of control animals fed lactalbumin diet, although they were less than those in rats given raw EMP-175 or PROC beans (Figure 1). The crypts of Lieberkuhn in the jejunum of rats fed diets containing any of the three raw bean cultivars were deeper than those from similar samples of cooked bean or lactalbumin controls (Table V). Consumption of raw RAZ-2 or EMP-175 beans but not of PROC beans reduced villus height in the jejunum (Table V).

Pancreas enlargement was evident in rats fed with all of the raw bean diets (Figure 1). Raw EMP-175 or PROC beans, but not the raw RAZ-2 diet, also significantly reduced the weight of the thymus and the gastrocnemius hind-limb muscles of rats.

Heat treatment of RAZ-2, EMP-175, or Processor beans (results for PROC not given), by boiling fully hydrated seeds at 100 °C for 10 min, abolished most, but not all, of their deleterious effects on body metabolism (Tables III and IV). With unrestricted access to cooked bean diets, the appetite of the rats improved considerably. Furthermore, the weight gain of rats given diets containing any

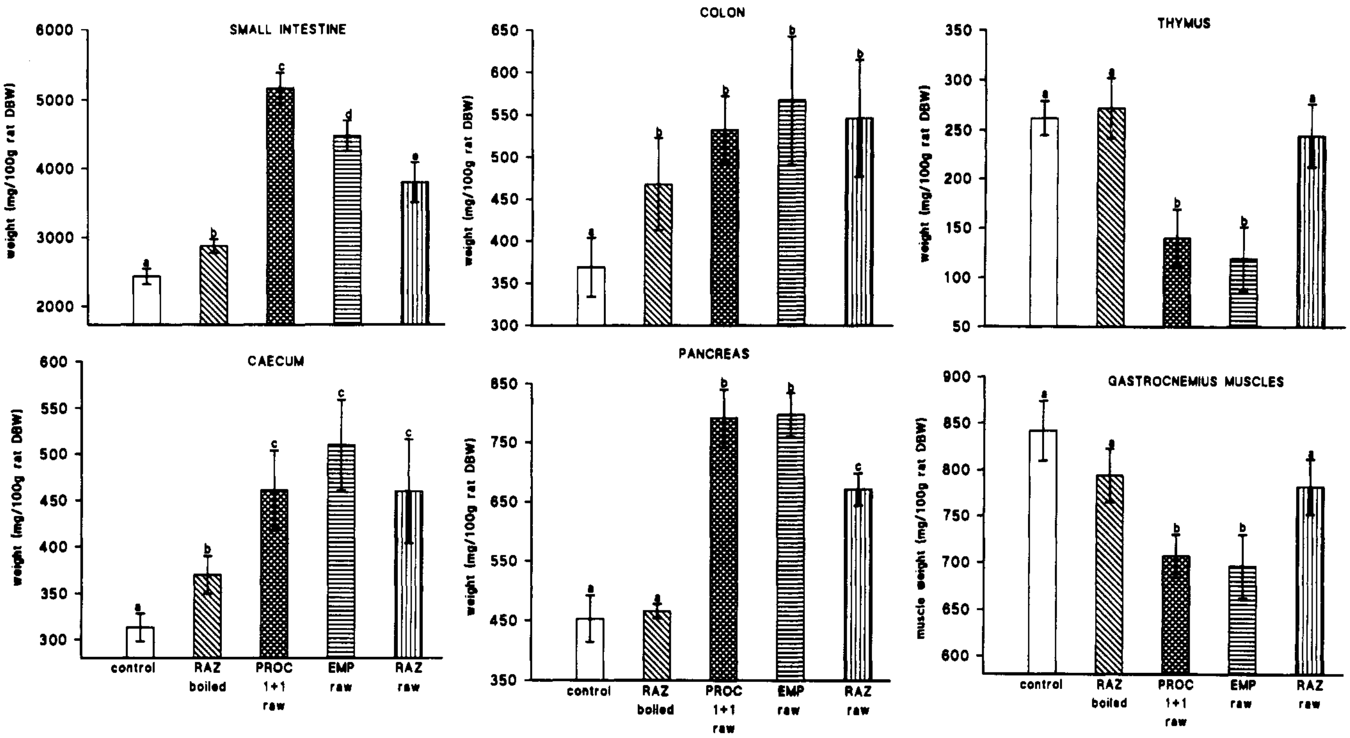

Figure 1. Weight of the small intestine, caecum, colon, pancreas, thymus, and hind leg gastrocnemius muscles of rats fed on lactalbumin (control) or on diets containing bean meals (6 g/day per rat) for 10 days. Values for each parameter with distinct superscripts differ significantly ($P < 0.05$); $n = 8$ for all groups.

Table V. Villus Height and Crypt of Lieberkuhn Depth in Sections Taken from the Upper Jejunum of Rats Fed (6 g/Day per Rat) on Diets Containing Bean Meals or Lactalbumin (Control) for 10 Days[a]

diet	villus height, μm	crypt depth, μm
2, 10% control protein	479 ● 10^{a}	119 ± 3^{a}
4, 10% raw RAZ-2 protein	445 ● 4^{b}	200 ± 3^{b}
6, 10% boiled RAZ-2 protein	442 ± 14^{b}	92 ± 3^{c}
5, 10% raw EMP-175 protein	387 ± 8^{c}	236 ± 6^{d}
3, 5% raw Processor protein + 5% control protein	508 ± 9^{a}	221 ± 7^{d}

[a] Values in a vertical row with distinct superscripts differ significantly ($P < 0.01$).

of the heat-treated bean seeds on either restricted intake or ad libitum was close to the lactalbumin control values. In addition, their body nitrogen and lipid contents approached that of rats fed lactalbumin diet, and the weights of their pancreas, caecum, thymus, and gastrocnemius muscles were also similar to those of lactalbumin control animals (Figure 1). However, although the net protein utilization and the biological value of the diets made up with cooked beans of either of the three lines investigated (RAZ-2, EMP-175, and PROC) were similar, they were still significantly below that of the lactalbumin control. Moreover, although the trophic effects of the raw RAZ-2, EMP-175, or PROC bean diets on the rat small intestine were greatly reduced by prior heat treatment of the seeds (Figure 1), a small residual effect remained. Clearly, although the crypts of Lieberkuhn in jejunal samples from the animals given heat-treated beans were not enlarged (Table V), their small intestine was still slightly heavier than that of rats fed lactalbumin-based control diet (Figure 1). Similarly, the weight of the caecum and colon of rats fed heat-treated beans remained significantly elevated in comparison with that of rats on lactalbumin control diet (Figure 1).

DISCUSSION

Raw RAZ-2 beans, when given as the sole source of dietary protein, inhibited but did not fully prevent the growth of rats over the 10-day experimental period. In contrast, at a comparable level of dietary inclusion, PROC bean of high PHA content has been shown to be lethally toxic within 3–5 days (Pusztai and Palmer, 1977; Pusztai, 1989, 1991). Therefore, in comparison, raw RAZ-2 beans were considerably less toxic, so much so that these beans could be consumed by rats without explicit toxic effects even when they were the sole source of dietary protein, in contrast to the more common toxic varieties such as PROC. As raw RAZ-2 beans might also be consumed by higher animals including humans without major problems, the new line is superior to common bean varieties not only because of its higher insect resistance but also because it is far less toxic. It appears that this improvement in nutritional quality may have been due to the reduced content of PHA of the bean, probably as a result of the introduction of high levels of arcelin 1 into the seed of the new line. Indeed, the performance of rats given RAZ-2 was significantly better than that of rats given raw EMP-175, the insect-susceptible parent line which contains 7 times more toxic PHA but does not contain arcelin 1.

Raw RAZ-2 beans did, however, have a number of antinutritional effects on body metabolism which could have harmful consequences. In the case of rats, these included the stimulation of hyperplastic growth of the small intestine, enlargement of the pancreas, and decline in body lipid content. All of these effects, which commonly occur in rats consuming diets containing most *P. vulgaris* cultivars, have previously been shown to be mediated primarily by PHA in the beans, while the enlargement of the pancreas is induced by both dietary trypsin inhibitors and lectins (Liener, 1987; Pusztai, 1989, 1991). However, the changes in small intestine weight and body lipid content caused by consumption of the raw RAZ-2 diet appeared to be significantly higher than could be accounted for by the low intake of PHA from the diet. Indeed, the increase in crypt length induced by RAZ-2 bean, which is a good indicator of small intestinal growth, was only slightly below that induced by either raw EMP-175 or PROC diets, but its PHA content was a fraction of that of the other two cultivars (Table II). This may indicate that other components in the beans, possibly arcelin 1 which has some similarities to lectins, were involved in the slight antinutritive effects of the raw RAZ-2 bean.

Most of the antinutritional effects of RAZ-2 beans could be abolished by boiling fully hydrated beans at 100 °C for 10 min, since arcelins, like PHA and the trypsin inhibitors, are heat-labile (Cardona et al., 1990; Grant et al., 1982; Liener, 1987). However, a slight increase in the weight of the small intestine, caecum, and colon was still evident in rats fed heat-treated RAZ-2 beans. These residual effects were also evident with heat-treated EMP-175 or Processor beans (results not given) and may possibly be due to increased mucus production or other bulk-related enlargement of the gut caused by heat-resistant components of the beans (Wyatt et al., 1988).

CONCLUSIONS

Diets containing raw RAZ-2 beans were essentially nontoxic for rats. However, since the digestion and utilization of bean proteins in the diet were depressed, the growth of the rats was poor. In addition, raw RAZ-2 beans had other deleterious effects on body metabolism, including stimulation of the growth of both small intestine and pancreas and loss of body lipid, although these effects were far less than those observed with other raw beans of high PHA content. As the level of toxic PHA in RAZ-2 bean was low and not sufficient to account for the slight antinutritive effects, other dietary components, possibly arcelin 1, could have contributed to the low nutritional value of the raw seedmeal of this new insect-resistant cultivar. Despite this, RAZ-2 bean has now been shown to be superior to its parental line or other bean cultivars not only because the insect resistance of this bean is high but also because it is not toxic even when eaten raw. Finally, as expected, most of the antinutritional effects of RAZ-2 beans, like those of other bean lines, could be abolished by rigorous heat treatment of the beans. However, the utilization of bean proteins in the diet and their biological value, even after cooking, were still low compared with high-quality animal proteins.

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LITERATURE CITED

Cardona, C.; Posso, C. E.; Kornegay, J.; Valor, J.; Serrano, M. Antibiosis effects of wild dry bean accessions on the mexican bean weevil and the bean weevil (Coleoptera: Bruchidae). *J. Econ. Entomol.* **1989**, *82*, 310–315.

Cardona, C.; Kornegay, J.; Posso, C. E.; Morales, F.; Ramirez, H. Comparative value of four arcelin variants in the development of dry bean lines resistant to the mexican bean weevil. *Entomol. Exp. Appl.* **1990**, *56*, 197–206.

Cardona, C.; Dick, K.; Posso, C. E.; Ampofo, K.; Nadhy, S. M. Resistance of a common bean (Phaseolus vulgaris L.) cultivar to post-harvest infestation by Zabrotes subfasciatus (Boheman) (Coleoptera: Bruchidae) 2 storage test. *Trop. Pest Manage.* **1992**, *38* (2), 173–175.

Chrispeels, M. J.; Raikhel, N. Lectins, lectin genes and their role in plant defense. *Plant Cell* **1991**, *3*, 1–9.

Coates, M. E.; Donaghue, P. N.; Payne, P. R.; Ward, R. J. In *Laboratory Animals Handbooks 2*, Dietary standards for laboratory rats and mice; Coates, M. E., Donaghue, P. N., Payne, P. R., Ward, R. J., Eds.; London Laboratory Animals Ltd.: London, 1969; pp 13–15.

Davidson, J.; Mathieson, J.; Boyne, A. W. The use of automation in determining nitrogen by the kjeldhal method with final calculations by computer. *Analyst* **1970**, *95*, 181–193.

Grant, G.; More, L. J.; McKenzie, N. H.; Pusztai, A. The effect of heating on the haemagglutinating activity and nutritional properties of bean (*Phaseolus vulgaris*) seeds. *J. Sci. Food Agric.* **1982**, *33*, 1324–1326.

Grant, G.; More, L. J.; McKenzie, N. H.; Stewart, J. C.; Pusztai, A. A survey of the nutritional and haemagglutination properties of legume seeds generally available in the UK. *Br. J. Nutr.* **1983**, *50*, 207–214.

Grant, G.; McKenzie, N. H.; Watt, W. B.; Stewart, J. C.; Dorward, P. M.; Pusztai, A. Nutritional evaluation of soybeans (*Glycine max*): Nitrogen balance and fractionation studies. *J. Sci. Food Agric.* **1986**, *37*, 1001–1010.

Harmsen, R.; Bliss, F. A.; Cardona, C.; Posso, C. E.; Osborn, T. C. Transferring genes for arcelin protein from wild to cultivated beans: implications for bruchid resistance. *Annu. Rep. Bean Improv. Coop.* **1988**, *31*, 54–55.

Liener, I. E. Nutritional significance of lectins in the diet. In *The Lectins*; Liener, I. E., Sharon, N., Goldstein, I. J., Eds.; Academic Press: New York, 1987; pp 527–552.

Lioi, L.; Bollini, R. Identification of a new arcelin variant in wild bean seeds. *Bean Improv. Coop.* **1989**, *32*, 28.

Minney, B. H. U.; Gatehouse, A. M. R.; Dobie, P.; Dendy, J.; Cardona, C.; Gatehouse, J. A. Biochemical bases of seed resistance to *Zabrotes subfasciatus* (bean weevil) in *Phaseolus vulgaris* (common bean): a mechanism for arcelin toxicity. *J. Insect Physiol.* **1990**, *36*, 757–767.

Osborn, T. C.; Blake, T.; Gepts, P.; Bliss, F. A. Bean arcelin. Genetic variation, inheritance and linkage relationships of a novel seed protein of *Phaseolus vulgaris* L. *Theor. Appl. Genet.* **1986**, *71*, 847–855.

Osborn, T. C.; Alexander, D. C.; Sun, S. S. M.; Cardona, C.; Bliss, F. A. Insecticidal activity and lectin homology of arcelin seed protein. *Science* **1988a**, *240*, 207–210.

Osborn, T. C.; Burow, M.; Bliss, F. A. Purification and characterization of arcelin seed protein from common bean. *Plant Physiol.* **1988b**, *86*, 399–405.

Palmer, R.; McIntosh, A.; Pusztai, A. The nutritional evaluation of kidney bean (*Phaseolus vulgaris*). The effect on nutritional value of germination and changes in trypsin inhibitor content. *J. Sci. Food Agric.* **1973**, *24*, 937–944.

Pusztai, A. Biological effects of dietary lectins. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*; Huisman, J., van der Poel, T. F. B., Liener, I. E., Eds.; Pudoc: Wageningen, The Netherlands, 1989; pp 17–29.

Pusztai, A. *Plant Lectins*; Cambridge University Press: Cambridge, U.K., 1991.

Pusztai, A.; Palmer, R. Nutritional evaluation of kidney bean *Phaseolus vulgaris*. The toxic principle. *J. Sci. Food Agric.* **1977**, *28*, 620–623.

Wyatt, G. M.; Horn, N.; Gee, J. M.; Johnson, I. T. Intestinal microflora and gastrointestinal adaptation in the rat in response to non-digestible dietary polysaccharides. *Br. J. Nutr.* **1988**, *60*, 197–207.

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