Effect of Different Heat Treatments on the Antinutritional Activity of *Phaseolus vulgaris* (Variety Ojo de Cabra) Lectin

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Ojo de Cabra bean lectin was partially characterized, and the nutritive value of raw and heat-processed beans were investigated in relation to this compound. The lectin purified by affinity chromatography on immobilized fetuin presented two bands of about 30 kDa in SDS–PAGE. It had an unusual high hemagglutinating capacity, and it was inhibited by fetuin and galactose. The lectin activity decreased 84% by dry heating and 99.8% by cooking. Although biological values of cooked beans were similar to those of other beans, toasting affected the PER and weight of rats, but not the NPR. Raw beans were highly toxic; 33% of animals died at 10 days. The NPR value was negative, and this group lost more weight than the N-free diet group. The histological examination revealed that the dietary lectin was bound to the mucosal surface coat of the duodenum and jejunum.

INTRODUCTION

It is generally accepted that lectins constitute the main factor responsible for the dietary toxicity of raw *Phaseolus vulgaris* beans (Lafont et al., 1988). A number of researchers have reported that the toxic effects could be prevented by boiling hydrated beans for 10 min (Grant et al., 1982); others have found that heating some types of beans at 82 °C for 1 h failed to inhibit their toxicity (Coffey et al., 1985). Furthermore, Bender and Reaidi (1982) found that heating beans for 15–45 min at 80 °C increased hemagglutination activity about 5-fold.

These controversial results suggest that bean types vary in the amount and toxicity of their lectins (Koehler et al., 1986).

Phaseolus vulgaris variety Ojo de Cabra, a legume crop readily available in northwestern Mexico (Sonora), has had low acceptability for human consumption; however, its utilization for poultry and swine rations has increased. Currently, the avian industry in Sonora is using Ojo de Cabra bean flour to partially substitute soybean meal in poultry diets. A toasting process is the only treatment usually applied to the legume prior to its diet use.

In this context, nutritive value and protein quality for this product and its relationship to lectin content must be adequately evaluated.

Our investigation was undertaken to partially characterize the Ojo de Cabra lectin and to evaluate its effect on the protein nutritive value of toasted and cooked beans by using the rat as a model.

MATERIALS AND METHODS

Lectin Purification and Partial Characterization. Bean flour was suspended in 0.15 M NaCl at 10% (w/v) and stirred for 2 h; the pH was adjusted to 4 and extraction continued overnight. After centrifugation, the crude extract was run through an affinity chromatography column containing fetuin coupled at Mini-Leak (Kem-En-Tec) as previously described (Vázquez-Moreno et al., 1990). The crude extract and the sodium phosphate buffer (PBS) washing and eluted fraction with glycine hydrochloride, pH 2.5, were probed for hemagglutination with trypsinized erythrocyte (Allen et al., 1973). The protein concentration of fractions was determined according to the Lowry method (Lowry et al., 1951).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out according to Laemmli's technique (Laemmli, 1970) for crude extract and fractions. Specificity of the purified lectin was made by hemagglutination inhibition with sugars and glycoproteins commonly probed. Double immunodiffusion of purified bean lectin against rabbit anti-PHA (Sigma, St. Louis, MO) was carried out according to the Ouchterlony technique (Ouchterlony and Nilsson, 1975).

Sample Treatment. Beans were cooked in an open kettle in boiling water for 140 min according to regional customs in home bean processing for human consumption as reported by Goycoolea et al. (1990). The proportion was 1:5 beans:water; broth was separated, and the beans were dried in a forced convection oven at 56 °C for 18 h. The toasted beans were obtained directly from livestock producers as is fed to animals, and the toasted process specified by them was the use of a gas rotatory oven with dry heat set at a temperature of 135 °C for a period of 15 min.

Lectin Determination in Samples. Finely ground raw, toasted, and cooked beans were extracted as previously described under Lectin Purification Partial Characterization. Fresh feces were homogenized with saline solution and clarified by filtration; hemagglutination activity was determined with glutaraldehyde-treated erythrocytes (Turner and Liener, 1975). Hemagglutination titers and protein concentration of crude extracts were determined for the bean samples. Specific activity was calculated as hemagglutination titer per protein concentration.

Biological and Histological Evaluations. Three independent experiments were utilized to evaluate the cooked, toasted, and raw bean treatments but with identical protocols and ANRC–casein control diets on each occasion. Diets for all feeding trial experiments were designed according to the AOAC protocol for the protein efficiency ratio (PER) (AOAC, 1984). All sources were previously analyzed for proximate composition and individual diets formulated to contain 10% protein at equal energetic densities. Table I shows the composition of the basal diet. The test diets were formulated from raw, toasted, and cooked beans. The control diet was ANRC–casein (Bioserv), and an extra nitrogen-free treatment was utilized for NPR evaluation.

Protein Efficiency Ratio and Net Protein Ratio. Six weanling male Sprague-Dawley rats were randomly assigned per treatment and housed individually in stainless steel cages with feed and deionized water provided ad libitum during 28 days for PER and 10 days for NPR. Animals were housed under controlled environmental conditions at 26 ± 1 °C and 55–65% relative humidity and a 12-h light and dark cycle.

Histological Studies. After 10 days, the animals fed the raw beans were sacrificed; gastric and intestinal tissues were immediately dissected and fixed in 8% formaldehyde for 24 h. Tissues embedded in paraffin were cut at 5 μm, deparaffinized, and routinely stained with hematoxylin–eosin (H–E) (Luna, 1968). Similar specimens were rinsed with PBS containing 1% bovine serum albumin (BSA) for lectin detection. The sections were incubated for 30 min at room temperature in a diluted solution

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Table I. Composition of the Basal Diet Used for in Vivo Evaluation of Experiment Formulations[a]

ingredient[b]	%	ingredient	%
corn oil	8.0	cellulose	1.0
vitamin premix[c]	1.0	water	5.0
mineral premix[d]	5.0	protein source	10.0
Cr_2O_3	0.2	starch and dextrose to make 100% of diet	–

[a] Corn oil, mineral premix, cellulose, and water were adjusted after proximate analysis of ingredients. Sample was calculated to contain 1.6% N in diet (10% protein), according to AOAC method 43.253, which is applicable to materials with %N above 1.8. [b] All the ingredients except corn oil were from Bioserv, Inc. [c] The vitamin premix supplied the following (g/kg of diet): ascorbic acid 0.45; biotin 0.0002; calcium pantothenate 0.03; choline 0.633; folic acid 0.0009; inositol 0.05; menadione 0.02; niacin 0.04; PABA 0.05; pyridoxine 0.01; riboflavin 0.01; thiamin 0.01; vitamin A 9000 IU; vitamin B 120.01 mg; vitamin D 1000 IU; vitamin E 25 IU. [d] Mineral premix supplied the following (g/kg of diet): aluminum 0.0005; calcium 11.0865; chlorine 4.7935; copper 0.0175; fluoride 0.0027; iodine 0.0030; iron 0.385; magnesium 0.3812; manganese 0.0055; phosphorus 2.5305; potassium 5.8820; sulfur 0.1162; zinc 0.0637.

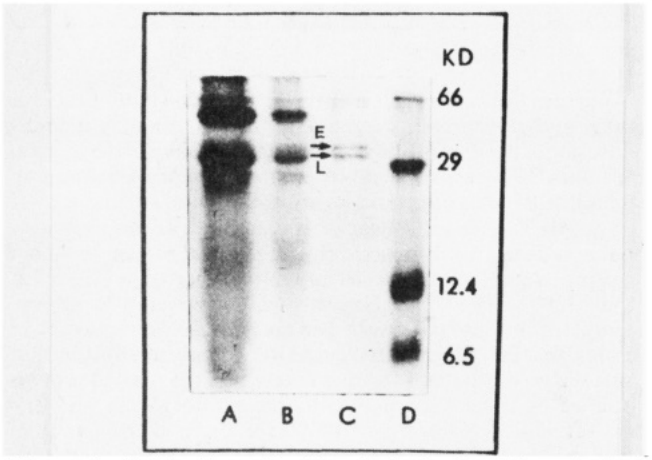

Figure 1. SDS–polyacrylamide gel electrophoresis analysis of purified Ojo de Cabra bean lectin. (A) Crude extract; (B) unbound fraction; (C) bound fraction; (D) molecular weight standards.

of rabbit anti-PHA, rinsed in PBS containing 1% BSA, and incubated for another 30 min with appropriately diluted FITC-conjugated goat anti-rabbit IgG, rinsed in PBS and mounted in buffered glycerol saline solution.

Sections stained with H–E were examined by using a light microscope and sections stained with FITC-conjugated anti-IgG with a Zeiss microscope equipped with an Osram HBO-200 lamp.

Statistical Analysis. Relative PER and NPR results in experiments I (cooked beans) and II (toasted beans) were analyzed according to Student's *t*-test.

RESULTS AND DISCUSSION

Ojo de Cabra's Bean Lectin Properties. Previously, lectins of others *P. vulgaris* beans have been purified by affinity chromatography on immobilized fetuin (Lafont et al., 1988; Donatucci et al., 1987). As *P. vulgaris* type 1 Processor (Pusztai et al., 1983), Ojo de Cabra beans contain two basic types of lectin subunits of about 30 000 Da, designated E and L (Figure 1). Furthermore, Ojo de Cabra lectin is structurally related to erythroagglutinin E from red kidney bean as observed by immunodiffusion (figure not shown). In inhibition assays the hemagglutination of the purified lectin was strongly inhibited by 1% fetuin and weakly inhibited by 0.1 M galactose but did not interact with GalNAc (Table II). As far as we know, there is no other *Phaseolus* lectin with this specific interaction pattern. *Phaseolus calcaratus* lectin II reacts specifically with galactose and GalNAc, but its electrophoretic pattern is very different (Datta et al., 1988). Lima bean lectin interacts weakly with galactose but better with GalNAc (Goldstein and Poretz, 1986), and it has blood group specificity (Pusztai et al., 1983). Ojo de Cabra's lectin does not have blood specificity.

Table II. Inhibition, by Sugars and Fetuin, of the Hemagglutinating Activity of the Ojo de Cabra Bean Lectin

compd[a]	hemagglutination titer	compd[a]	hemagglutination titer
none (control[b])	256	D-glucose	256
D-galactose	64	L-fucose	256
N-acetyl-D-galactosamine	256	D-mannose	256
		fetuin	4
N-acetyl-D-glucosamine	256		

[a] Concentration of sugars was 0.1 M and fetuin at 1%. [b] Lectin concentration was 0.212 mg/mL.

Table III. Lectin Activity of Ojo de Cabra Beans with Different Heat Treatments As Compared with That of the Raw Beans

sample	sp act./g of sample
raw beans	367.4
toasted beans	57.6
cooked beans	0.8

Ojo de Cabra's Lectin Content in Heat-Treated Samples. Table III shows that the bean lectin loses biological activity with the different heat treatments. Previously, it has been shown that soybean active lectin presents an inverse relationship to treatment during food processing (Calderón de la Barca et al., 1991). Other studies have shown that lectins can be inactivated to a considerable extent during cooking but dry heating does not reduce the activity; presoaking and autoclaving are the best treatments for seed lectin inactivation (Ayyagari et al., 1989). In addition, the lectin activity was resistant to gastrointestinal proteolysis as shown by a hemagglutination titer of 16 in the saline extract of feces from rats fed the raw bean diet.

Histological Evaluations. Macroscopic examination of the gastrointestinal tract indicated the appearance of inflammation in the rats that were exposed to raw Ojo de Cabra beans. In other studies, in addition to inflammation, Bulajic et al. (1986) found some exudation in the gastrointestinal tract using a similar level of raw kidney bean in the diet. Our findings did not detect other disorders such as diarrhea in the experimental animals.

Histological observations with a light microscope showed the following morphological alterations in the duodenum of rats fed the raw bean diet: (a) inflammatory process; (b) mitosis of the epithelial cells; and (c) bacterial adherence on the tips of the villi (Figure 2). Similar alterations were observed in the jejunum.

When beans have been supplied for short terms, other authors have found gross morphological injuries (Oliveira et al., 1989). By contrast, in our experiment the density of the goblet cells, crypts, and villi were the same as in the controls. Our results were similar to those for the raw lima bean fed rats reported by Lafont et al. (1988).

Indirect immunohistochemistry revealed that dietary lectin of raw beans was bound to the mucosal surface coat of the duodenum and jejunum (Figure 3). Traces of lectin were detected on the mucosal coat of the gastric epithelium and ileum. This pattern of binding is the same that was observed by Bulajic et al. (1986) with kidney bean.

Biological Evaluation. The PER values obtained for the different process treatments are shown in Table IV. Cooked beans had a PER value of 1.33, similar to that reported by other authors for *P. vulgaris* cultivars cooked

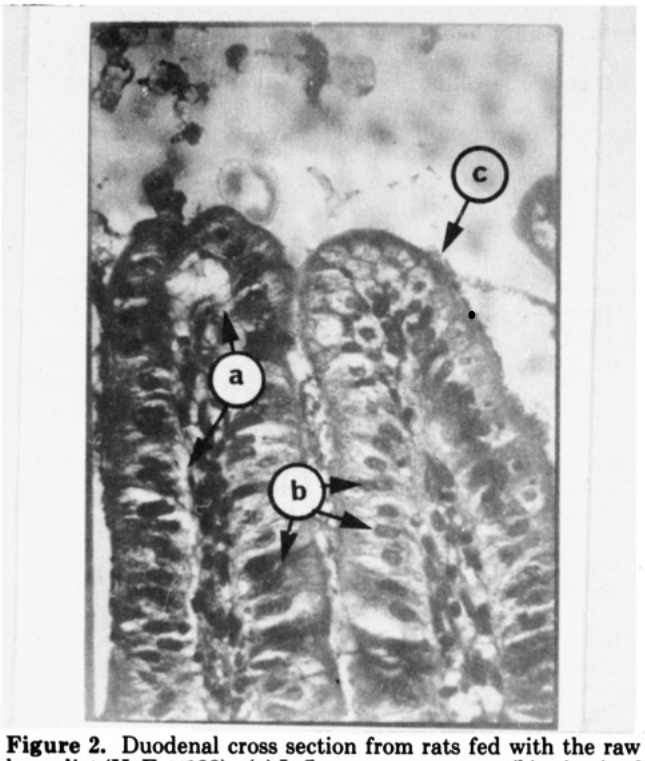

Figure 2. Duodenal cross section from rats fed with the raw bean diet (H–E, ×128). (a) Inflammatory process; (b) mitosis of the epithelial cells; (c) bacterial adherance.

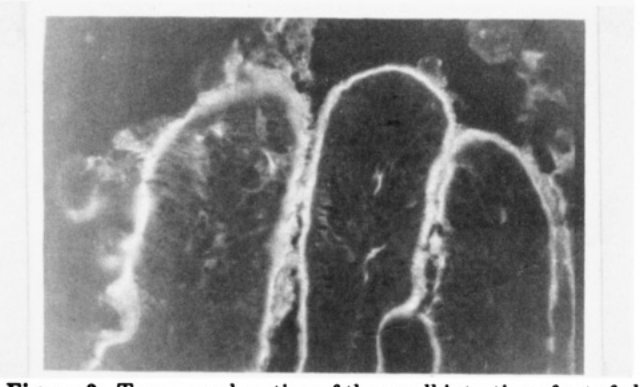

Figure 3. Transversal section of the small intestine of rats fed with a raw bean containing diet. Sections were treated with rabbit anti-PHA antibodies followed by goat anti-rabbit IgG–FITC.

Table IV. Protein Nutritive Value by Various Parameters

	biological response[a]				
	10-day assay			28-day assay	
source	NPR	R-NPR,[b] %	wt, g	PER	R-PER,[b] %
expt I					
cooked beans	2.49	55.0[a]	6.4	1.33	48.0[a]
ANRC–casein II	4.55	100.0	33.6	2.77	100.0
expt II					
toasted beans	1.91	53.0[a]	4.1	0.36	14.9[b]
ANRC–casein I	3.63	100.0	28.0	2.42	100.0
expt III					
raw beans	−1.1		−14.7	ND[c]	ND
ANRC–casein III	4.1	100.0	36.6	2.62	100.0
N-free diet			−10.5	ND	ND

[a] Values with different superscripts are significantly different ($p < 0.001$). [b] R-PER and R-NPR values are relative values to the casein control, expressed as percentages. [c] ND, not determined.

under similar conditions (Goycoolea et al., 1990). However, the PER for the toasted beans was significantly lower ($p < 0.001$) than that obtained by cooking in water. On the other hand, it was not possible to evaluate a PER for the raw beans, since at 10 days there was 33% mortality and the rest of the animals were in an obvious acute toxicological condition.

Net protein ratio (Table IV) results in the toasted and cooked bean treatments did not show the same pattern as the PER experiments after standardizing for its casein control value. This could mean that theoretical nitrogen retention was not affected in a 10-day period in the toasted treatment and was sufficient for maintenance; however, in the long run the toasted beans eventually exert their toxicity and will not support growth as was observed by the protein efficiency ratio and reflected by the Lectin activity in the different heat treatments (Table III).

In addition, this group lost 40.6% more body weight than rats on a nitrogen-free diet (14.7 vs 10.5 g). These data agree with the studies reported by Oliveira et al. (1989), where a diet containing raw beans resulted in an average weight loss of 16.3 g in a period of 11 days. Furthermore, Koehler et al. (1986) reported weight losses greater than for nonprotein controls in rats fed with 7 cultivars of *P. vulgaris*, 5 groups with equal losses and 12 varieties that induced less weight loss. This also suggests that the toxic effect of Ojo de Cabra lectin ranks in the higher levels.

During the first 4 days the animals in the raw bean group showed an unusual aggressive behavior and progressively decreased their activity to practically immobility. There was desquamation of nose and skin in various areas of the body including the legs. Hair was opaque and rigid; the eyes were also dull and appeared to be sunken and half-closed almost permanently. There was tremor and mucous secretion principally from the nose. It is possible that in addition to the toxic effects the absorption of other nutrients different from nitrogen could be impaired by the lectin bound to the intestinal coat surface and that these symptoms are a combination of toxicity and malnutrition.

Under practical conditions people in the animal industry should be very cautious in the type of heat treatments applied before investing in and utilizing these type of legumes in their formulations with the potential losses in production. Even when water cooking methods are used, control of temperature and times must be considered carefully.

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