In Vivo Digestibility of Bean (*Phaseolus vulgaris* L.) Proteins: The Role of Endogenous Protein

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The effects of ingestion of diets containing cooked whole beans, albumins, or globulins on the fecal N and S origin were evaluated in rats by employing ^{15}N- and ^{35}S-labeled beans. The ingestion of whole beans caused the highest N and S excretion, mostly originated from exogenous sources. Whole beans and, to a lesser extent, albumins also increased metabolic fecal nitrogen excretion which represents approximately 20% of total N elimination. Isolated cooked bean globulins are well digested, having no effect on metabolic protein output. This suggested that other, nonprotein components present in whole beans may be involved in the increase of both exogenous and endogenous fecal protein.

INTRODUCTION

The low nutritional value of dried bean (*Phaseolus vulgaris* L.) proteins is attributed to low content and limited availability of essential amino acids (Evans and Bauer, 1978; Hernandez-Infante et al., 1979), to the presence of heat stable (Bressani et al., 1982; Deshpande et al., 1982) and residual heat labile antinutritional factors, or to poor protein digestibility (Sgarbieri and Whitaker, 1982; Thompson and Gabon, 1987).

The in vitro and in vivo digestibility of bean protein in rats is about 70% (Bressani and Elias, 1984; Durigan et al., 1987). In humans, bean protein digestibility is lower, reaching no more than 60% of the ingested nitrogen (Navarrete and Bressani, 1981; Bressani et al., 1982).

Cooking may improve the nutritional value of beans by eliminating some of the antinutritional factors, but protein digestibility remains poor. Under some conditions cooking may reduce protein digestibility. Lanfer Marquez and Lajolo (1981) reported a decrease in digestibility of isolated bean albumins on cooking.

The poor digestibility of beans has several causes: (1) formation of enzyme-resistant protein–tannin or protein–phytate complexes (Artz et al., 1987; Aw and Swanson, 1985); (2) direct partial inhibition of digestive enzymes by phenolics or phytates (Thompson et al., 1984: Singh, 1984); (3) unfavorable protein conformation or sequence of amino acids in the protein (Chang and Satterlee, 1981); (4) protein–protein or protein–carbohydrate interactions during thermal treatment (Semino et al., 1985). Protein digestibility is also related at the intestinal level to the indirect action of beans that leads to an increased excretion of endogenous body nitrogen. Bender and Mohammadiha (1981) and Sandaradura and Bender (1984) observed increased excretion of DNA and N from rats fed cooked beans and attributed the increases to an increased rate of mucosal cell turnover. On the contrary, Fairweather-Tait et al. (1983) observed only a moderate exfoliation of intestinal mucosal cells and concluded that the high N excretion was due to indigestible carbohydrates increasing the activities of the intestinal flora, leading to greater fecal N concentrations.

The objective of this research was to clarify the origin of fecal N and S in rats fed cooked whole beans or albumins and globulins extracted from the beans. ^{15}N- and ^{35}S-labeled beans were employed to separate exogenous (undigested dietary protein) from endogenous protein (residues of enzymic secretions, intestinal mucosal cell lining, and bacteria) elimination. Conventional methodology used previously did not consider specific metabolic absorption and secretion processes that occur in the gastrointestinal tract and significantly alter the composition of fecal nitrogen.

MATERIALS AND METHODS

Labeling of Beans. Bean seeds (*P. vulgaris* L.) of Carioca cultivar were grown under controlled conditions, using $^{15}NH_4Cl$ (10.5% of N as ^{15}N) and $Na_2{}^{35}SO_4$ (0.5 mCi) as fertilizers. The leaves received five additional sprays of $^{15}NH_4Cl$ in a 1% solution 5 weeks after planting. The beans were harvested 3 months after planting, dried, selected, and stored in a freezer.

Fractionation of Bean Proteins. The dried beans were ground to flour and passed through a 0.250-mm sieve; albumins were extracted with water and globulins with 0.5 M NaCl containing ascorbic acid (0.25 M) as previously described (Lanfer Marquez and Lajolo, 1981).

Thermal Treatment. Bean protein fractions were autoclaved at 121 °C for 30 min, at a flour to water ratio (w/v) of 1:5 for whole bean and 1:10 for the isolated protein fractions. Autoclaved beans with cooking water were dried in a circulating air oven and reground to pass a 60-mesh sieve. The protein fractions were lyophilized.

Biological Experiment. Diets. Rats were fed a powdered 10% protein diet containing either the whole bean flour or the isolated bean proteins. The compositions of the experimental diets are presented in Table I.

Experimental Design. The study was assessed by evaluating simultaneously ingestion and fecal excretion of total nitrogen, ^{15}N, total sulfur, and ^{35}S for each diet.

Six female (23 days old) Wistar rats were weighed and housed individually in screen mesh bottomed metabolic cages and fed ad libitum on one of the three nonlabeled experimental diets during an adaptation period of 3 days.

At the end of the adaptation period the number of rats in each experimental group was reduced to four, which were then submitted to a balance period of 12 days. From day 1 to day 6 each rat received a double labeled protein diet identical in composition with adaptation nonlabeled diet. From day 7 to day 12 the rats again received the nonlabeled diets. The feces of each rat were collected every 24-h interval, beginning at the 12th hour after the first ingestion of the labeled diets and ending 12 h after the ingestion of the last nonlabeled diet. Feces were dried to constant weight at 90 °C and analyzed individually. Food consumption was recorded daily.

Protein Determination. Total N was determined by the micro-Kjeldahl method (AOAC, 1975). A conversion factor of 6.25 was used.

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Table I. Composition of Experimental Diets

ingredients	10% protein diets based on whole bean	albumins	globulins
protein source, %	40.9	15.3	11.4
cornstarch, %	45.6	71.2	75.1

[a] Diets were fed as powders, containing 8% corn oil, 1% cellulose powder (Avicel pH 101, FMC Corp., Philidelphia, PA), 3.5% mineral mixture, and 1% vitamin mixture (AIN-76 formulation; American Institute of Nutrition, 1977).

Table II. Nitrogen, Sulfur, ^{15}N, and ^{35}S Contents in Protein Sources and Diets

elements	whole bean	albumins	globulin Gl
protein sources			
total N, %	4.12 ± 0.08	11.31 ± 0.07	16.92 ± 0.07
total S, %	0.238 ± 0.007	0.511 ± 0.010	0.240 ± 0.001
^{15}N, % of ^{15}N atoms in excess	1.052 ± 0.026	1.124 ± 0.007	0.960 ± 0.005
^{35}S, dpm × 10^{-4}/g	22.44 ± 0.21	44.40 ± 0.42	21.42 ± 0.21
diets[a]			
total N, %	1.64 ± 0.02	1.60 ± 0.03	1.55 ± 0.03
total S, %	0.17 ± 0.01	0.14 ± 0.01	0.09 ± 0.01
^{15}N, % of ^{15}N atoms in excess	0.493	1.076	0.498
^{35}S, dpm × 10^{-4}/g	4.91	7.14	1.17

[a] Whole bean and globulin diets were prepared by mixing protein sources with 50% unlabeled protein sources.

Total Sulfur Analysis. Beans, isolated bean protein fractions, diets or feces were digested with nitric and perchloric acid (Krug et al., 1977). The resulting sulfate was determined turbidimetrically with barium chloride solution in an automated analytical system, equipped with a Varian spectrophotometer, Model 634 (Krug et al., 1983).

Radioactive Sulfur Counting. Total ^{35}S was determined after mineralization with nitric and perchloric acid (Krug et al., 1977). Aliquots of the radioactive solutions were counted by liquid scintillation spectrometry using the scintillation fluid described by Bray (1960) and a Beckman LS 100C liquid scintillation counter. Radioactivity was corrected for background, counting efficiency by the external standard method, and decay, when appropriate. Sufficient counts were accumulated so that the counting error was less than 2%.

Evaluation of ^{15}N Enrichment. The quantity of ^{15}N was evaluated by using a Varian mass spectrometer, Model Matt 230, according to the method of Proksch (1969). The concentrations were expressed as percent of ^{15}N atoms in excess, by subtracting natural ^{15}N (0.365%) from experimental values, or as milligrams of ^{15}N in excess (considering negligible the mass variation from ^{14}N to ^{15}N).

RESULTS AND DISCUSSION

Excretion Pattern of ^{15}N and ^{35}S. Efficiency of incorporation of ^{15}N and ^{35}S and amounts of total nitrogen and sulfur in the whole bean and in isolated protein fractions are presented in Table II.

Figure 1 shows the fecal excretion of ^{15}N of rats on labeled whole bean, albumin, or globulin diets during the entire 12-day experimental period. The amount of ^{15}N excreted in feces increased during the first 2 days of ingestion of labeled diets, reaching then an equilibrium in which relation between ^{15}N ingested and fecal excreted ^{15}N was approximately constant. This equilibrium, considered a steady state, was maintained until day 6 when the rats were again fed unlabeled diets.

The ^{15}N excreted during the steady-state period was 29.4% of the ingested for whole beans, 14.8% for albumins, and 3.5% for globulins, while the ^{35}S elimination in the equilibrium was higher, 32.4%, 18.7%, and 6.7%, respectively, for whole beans, albumins, and globulins. The fecal ^{35}S excretion pattern (Figure 2) was similar to the ^{15}N

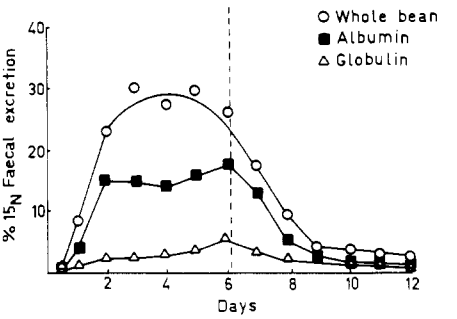

Figure 1. Fecal excretion of ^{15}N, expressed as percent of ingested ^{15}N.

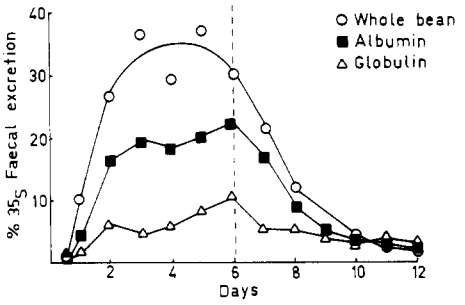

Figure 2. Fecal excretion of ^{35}S, expressed as percent of ingested ^{35}S.

Table III. Total Nitrogen and ^{15}N Digestibilities of Whole Bean and Isolated Protein Fractions

measure	protein source whole bean	albumins	globulins
intake			
total N, mg	93.35 ± 4.57	106.91 ± 10.97	70.84 ± 2.77
^{15}N, mg in excess	0.43 ± 0.02	1.16 ± 0.11	0.35 ± 0.14
fecal			
total N, mg	32.25 ± 3.30	21.46 ± 2.19	6.75 ± 1.87
^{15}N, mg			
12 h (exogenous)	0.04 ± 0.01	0.04 ± 0.02	trace
steady state (exogenous + endogenous)	0.13 ± 0.01	0.17 ± 0.02	0.02 ± 0.01
digestibility, %			
total N[a]	65.4 ± 4.0	79.5 ± 2.4	94.0 ± 3.6
^{15}N[b]	70.6 ± 2.8	82.2 ± 0.9	97.7 ± 1.6

[a] %N digestibility = [(dietary N − fecal N)/dietary N] × 100. Intake and output values are means ± SD (n = 4) over a 12-day period. [b] % ^{15}N digestibility = [(dietary ^{15}N − fecal ^{15}N)/dietary ^{15}N] × 100. Intake and output values are means ± SD (n = 4) for days 3–6 (steady state).

elimination, but there was a slightly higher excretion of ^{35}S, in relation to ^{35}S ingested, than for ^{15}N. Consequently, a tendency to lose more sulfur than nitrogen existed.

In combining the data representing separate determinations of total N and ^{15}N, calculated digestibility values are listed in Table III, together with ^{15}N excretion for 12 h after initiation of feeding the labeled diets. Apparent digestibility values calculated on the basis of total N over the entire experimental period were similar to previous data. We reported that the whole bean had the lowest protein digestibility (63%) followed by isolated bean proteins, such as glutelins (73%), albumins (79%), and globulins (89%) (Lanfer Marquez and Lajolo, 1990).

The ^{15}N present in the feces during the equilibrium (day 3 to day 6) was the sum of exogenous ^{15}N (ingested but not absorbed) plus the endogenous ^{15}N secreted in the intestinal lumen due to digestive enzymes, exfoliation,

Table IV. Total Sulfur and ^{35}S Digestibilities of Whole Bean and Isolated Protein Fractions

measure	protein source		
	whole bean	albumins	globulins
intake			
total S, mg	9.03 ± 0.48	9.18 ■ 0.84	ND[a]
^{35}S, dpm × 10^{-5}	2.62 ± 0.11	4.84 ± 0.43	0.77 ■ 0.20
fecal			
total S	2.45 ± 0.48	2.10 ± 0.51	ND[a]
^{35}S, dpm × 10^{-5}			
12 h (exogenous)	0.27 ± 0.15	0.18 ± 0.14	0.004
steady state (exogenous + endogenous)	0.87 ± 0.14	0.89 ■ 0.11	0.045 ± 0.02
digestibility, %			
total S[b]	73.20 ± 5.50	76.70 ± 4.90	ND[a]
^{35}S[c]	67.50 ± 6.80	81.20 ± 1.20	93.20 ± 1.00

[a] Total S amounts were too small for evaluation. [b] %S digestibility = [(dietary S − fecal S)/dietary S] × 100. Intake and output values are means ± SD (n = 4) over a 12-day period. [c] %^{35}S digestibility = [(dietary ^{35}S − fecal ^{35}S)/dietary ^{35}S] × 100. Intake and output values are means ± SD (n = 4) for days 3–6 (steady state) with exception of globulin diet, calculated from a pool of 4 rats.

bacteria, etc. Thus, ^{15}N excreted during the equilibrium permitted only calculation of protein apparent digestibility.

Data representing total sulfur and ^{35}S determinations 12 h after feeding and during the equilibrium are shown in Table IV. Apparent digestibilities of total sulfur and ^{35}S are calculated as for total N and ^{15}N.

To obtain more information about the individual contribution of exogenous undigested protein and endogenous protein formed in the intestine as a result of metabolic processes to the total fecal excretion, ^{15}N was evaluated 12 h after initiation of feeding the labeled diets. During the first 12 h it can be assumed that ^{15}N in the feces originates greatly from undigested exogenous ^{15}N-labeled proteins, without interference of labeled endogenous proteins; most of the endogenous protein, within the first 12 h, is synthesized in the prelabeling period (Zimmer et al., 1975; Zebrowska et al., 1976; Souffrant et al., 1981).

Exogenous ^{15}N in feces expressed as percentage of ingested ^{15}N was 9.1%, 3.2%, and 1%, respectively, for whole beans, albumins, and globulins. It can be seen that the ingestion of whole beans caused an excretion of exogenous N approximately 10 times higher than the excretion observed for isolated albumins and globulins; this may be due to a reduced digestibility of the whole bean proteins or even to an increased intestinal passage, rendering more difficult the amino acid absorption processes. Bean globulins caused the smallest exogenous N excretion (Table III).

It is important to stress that these values are only approximations because at the time used (12 h) not all the ^{15}N ingested had been eliminated in feces, and the values obtained can be considered only comparatively between the three protein sources; however, they help to show the relative difference in exogenous excretion existing among the different protein fractions and the effect of bean components on them.

After the same time (12 h), exogenous ^{35}S in feces expressed as percentage of the ingested ^{35}S was 9.9%, 4.3%, and 0.6%, respectively, for whole beans, albumins, and globulins.

Comparing the results obtained for ^{15}N and ^{35}S we may conclude they are not significantly different for each one

Table V. Isotopic Dilution of ^{15}N and ^{35}S in the Gastrointestinal Tract[a]

measure	diets		
	whole bean	albumins	globulins
^{15}N in diets, % of ^{15}N atoms in excess	0.493	1.076	0.498
^{15}N in feces (12 h), % of ^{15}N atoms in excess	0.125	0.185	0.028
dilution factor of ^{15}N[b]	3.9	5.8	17.8
^{35}S in diets, dpm × 10^{-5}/100 g of S	29.34	52.12	ND[c]
^{35}S in feces (12 h), dpm × 10^{-5}/100 g of S	11.64	9.00	ND
dilution factor of ^{35}S[b]	2.5	5.8	ND[d]

[a] Mean values of two or three determinations. [b] Dilution factor = ^{15}N or ^{35}S in diets/^{15}N or ^{35}S in feces. [c] ND, not determined. [d] ^{35}S dilution factor for globulins was not determined due to insufficient amount of total S.

of the protein sources, indicating both N and S have a similar level of digestibility.

Another way of looking at the exogenous excretion is calculating the isotopic dilution of fecal ^{15}N by endogenous unlabeled N. The higher the isotopic dilution, the higher is the elimination of endogenous N in feces. Table V gives the isotopic dilution of fecal ^{15}N by endogenous unlabeled N 12 h after the first ^{15}N ingestion. The feces of rats fed the whole bean diet contained a ^{15}N concentration diluted by only 3.9 times in relation to the ^{15}N concentration in the whole bean diet. Isotopic dilution calculated for the albumin- and globulin-fed rats was 5.8 and 17.8 times, respectively. Feeding of globulins produces low total fecal N elimination and high isotopic dilution, indicating most fecal N after feeding globulins is endogenous, while for the whole bean diet a significant part of the fecal N is represented by undigested exogenous proteins. The albumins produced an intermediary exogenous/endogenous fecal N. Dilution of ^{35}S in the feces after 12 h was 2.5 and 5.8 times for the whole bean and albumin diets, respectively, and compares well with ^{15}N dilution factors. The globulin diet was not determined because the amount of total sulfur was too small for a precise evaluation (Table V).

Endogenous ^{15}N and ^{35}S. After day 6 of the experiment, the rats were again fed unlabeled diets. Feces ^{15}N decreased sharply during the first 3 days (because the labeled ^{15}N diets ceased) and then more slowly. In the first 3-day decay period, most of label originated from undigested exogenous proteins eaten before day 6 plus the loss of undigested labeled metabolic proteins. At this time it is impossible to appreciate the extent of metabolic N loss.

In the slow decay phase (days 9–12) the ^{15}N and ^{35}S values in feces represented mostly the label originated from endogenous body proteins (Figure 3). When the ^{15}N curves (slow phase) were extrapolated to zero time (day 6), the amount of ^{15}N excreted, approximately 0.028 and 0.033 mg of ^{15}N for whole bean and albumins, respectively, represented 22% and 19% of ^{15}N eliminated in the steady state and was considered endogenous. The N elimination from rats fed globulins was very low and corresponded predominantly to endogenous sources, demonstrated by the ^{15}N excretion curve (Figure 3) which does not exhibit the two-phase response as observed for the whole beans and albumins.

Excretion of endogenous sulfur following feeding when labeled ^{35}S diets ceased is similar (Figure 4). Endogenous

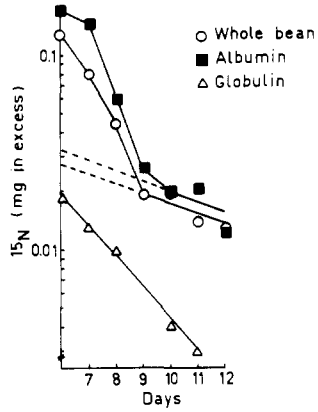

Figure 3. ^{15}N fecal excretion during the decay period when the rats ingested unlabeled diets.

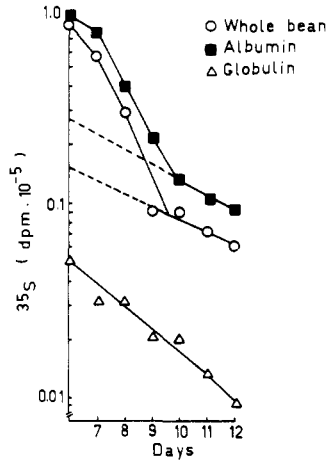

Figure 4. ^{35}S fecal excretion during the decay period when the rats ingested unlabeled diets.

eliminations of ^{35}S were 0.155, 0.250, and 0.050 dpm × 10^{-5} ^{35}S, respectively, for whole beans, albumins, and globulins, representing 18%, 27%, and 96%, respectively, of the ^{35}S eliminated in the steady state.

The results indicate that consumption of whole beans and, to a lesser extent, isolated bean albumins led to an increase in exogenous and endogenous N excretion. Contrarily, cooked bean globulins are well digested, having no effect on metabolic nitrogen loss.

The reasons for the enhanced N loss by whole beans are not clear, but interactions of bean protein with such nonprotein bean components as fiber, carbohydrates, and/or tannins during cooking are probable. Also, bean albumins are not pure proteins but a fraction containing more than 30% carbohydrate (Lanfer Marquez and Lajolo, 1990), and further studies are necessary to elucidate the effects of heating conditions on interactions of bean proteins with nonprotein bean components.

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