Relative Importance of Phytohemagglutinin (Lectin) and Trypsin-Chymotrypsin Inhibitor on Bean (*Phaseolus vulgaris* L.) Protein Absorption and Utilization by the Rat

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**Summary** The main objective of this work was to perform a comparative study of the antinutritional and/or toxic properties of phytohemagglutinin and trypsin-chymotrypsin inhibitor extracted from the seed of a commercial cultivar of edible bean used in Brazil. Bean proteins were extracted in acidic salt solution and fractionated by dialysis and centrifugation, then freeze-dried. The total freeze-dried bean extract and the globulin or albumin protein fraction were resuspended in distilled water and heated (100°C, 30 min) for inactivation of hemagglutinin. Diets were prepared with unheated bean protein fractions and heated ones (100% trypsin inhibitor activity, but 0% phytohemagglutinin activity). As a result, the inhibition of growth and poor dietary protein utilization were observed in rats fed diets containing unheated bean protein fractions, but not in rats fed diets containing heated fractions. It was thus assumed that phytohemagglutinin is the main antinutritional and toxic factor in dry bean (*Phaseolus*) protein and that trypsin inhibitor (Bowman-Birk type) did not interfere with rat growth.

**Key Words** dry bean, antinutritional factor, toxicity, phytohemagglutinin (lectin), trypsin inhibitor

The antinutritional action of trypsin inhibitor on rats fed raw soybean was first reported by Osborne and Mendel (1) almost a century ago. Soon it was demonstrated that this inhibitory action could be eliminated by heating (2, 3). Then a second class of legume seed proteins, phytohemagglutinin, was disclosed that selectively agglutinated erythrocytes in vitro, initiated mitosis in cell cultures of lymphocytes, and inactivated tumor cells (4). Trypsin-chymotrypsin inhibitors and phytohemagglutinins (lectins) are protein present in a variety of legume seeds. They are believed to have antinutritional properties by interfering with the growth and

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utilization of dietary nutrients (5-7). Besides the reported growth inhibitory action of trypsin-chymotrypsin inhibitors, legume seed lectins have been of great concern because of their toxicity (8-10). Some comprehensive reviews have been published on the nutritional and antinutritional properties of common bean proteins (11, 12). In spite of almost a century of investigation on the importance of trypsin-chymotrypsin inhibitors and phytohemagglutinins in the nutritive value and toxicity of legume seeds, many questions remain to be answered.

This paper will report on the nutritive value and toxicity of various bean protein fractions submitted to heat treatment in which the trypsin-chymotrypsin inhibitory activity has been preserved and the phytohemagglutinin (lectin) activity has been either preserved or completely inactivated.

MATERIALS AND METHODS

Materials. Raw bean seeds (Phaseolus vulgaris L.) of the cultivar “IAC Carioca 80-SH” were obtained from a special cultivation for use in this study.

Preparation of samples: The procedures for extraction and fractionation were according to Whitaker and Sgarbieri (13).

The bean flour (70 mesh) was suspended (1:10 w/v) in 0.5 M NaCl solution, adjusted to pH 2.5 with 0.1 N HCl solution and stirred continuously for 2 h at room temperature. The suspension was centrifuged (10,300 × g, 40 min, 4 °C), and the insoluble residue was discarded. The supernatant was submitted to dialysis against distilled water for 72 h at 4 °C, then centrifuged (10,300 × g, 40 min, 4 °C). The precipitate (globulin fraction) and the supernatant (albumin fraction) were then dehydrated by freeze-drying. A portion of the dialyzed raw extract was freeze-dried without centrifugation, and the resulting residue was used as total protein concentrate containing albumin and globulin. The protein isolated was resuspended in deionized water (1:5 w/v), heated at 100 °C for 30 min, then immediately cooled in an ice bath, followed by lyophilization. The dehydrated samples were kept in a freezer (−20 °C) before use.

Analytical procedures

Assays: Crude protein was determined by the semimicro-Kjeldahl method (% N × 6.25) procedure described in the AOAC (14). Trypsin inhibitor activity was measured by the method of Kakade et al (15) by the use of N-α-benzoyl-DL-arginine-p-nitroanilide (BAPNA) as substrate. A unit of trypsin activity was defined as a change of 0.01 unit absorbance at 410 nm and the activity of trypsin inhibitor expressed as units of trypsin inhibited (UTI) per milligram of protein (UTI/mg P). Phytohemagglutinin activity was measured by using trypsinized rabbit erythrocytes as described by Junqueira and Sgarbieri (16). The hemagglutinating titer was taken from the reciprocal of the highest dilution still capable of producing hemagglutination. The hemagglutinating activity (HA) was expressed as hemagglutinating titer per milligram of sample or protein (HT/mg P).

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Table 1. Composition of diets used in the rat assays.

| Diet components       | Percent by weight |
|-----------------------|-------------------|
| Protein\(^a\)         | 10.0              |
| DL-Methionine\(^b\)   | 0.2               |
| L-Cysteine\(^b\)      | 0.2               |
| Soybean oil           | 8.0               |
| Mineral mixture\(^c\) | 3.5               |
| Vitamin mixture\(^d\) | 1.0               |
| Cellulose powder      | 2.0               |
| Carbohydrate          | to 100.0          |

\(^a\) Ten percent was made up of 6% casein and 4% unheated bean protein, or 5% casein and 5% heated bean protein.

\(^b\) Methionine and cysteine were added to the mixture on the basis of 2% of dietary protein.

\(^c\) Mineral mixture according to Bieri et al (17).

\(^d\) Vitamin mixture, according to Reeves et al (18).

**Animal experiment**

**Preparation of diets:** Diets were prepared according to the basic composition presented in Table 1 (17, 18). Protein concentration was aimed at 10%, with casein providing 60% and 50% for the diets containing unheated and heated bean protein, respectively. Before mixing with casein, the bean protein was complemented with 2 g of DL-methionine plus 2 g of L-cysteine per 100 g of bean protein in order to compensate for the deficiencies of these amino acids in bean protein. A diet was also prepared in which trypsin-chymotrypsin inhibitor purified by affinity chromatography (trypsin-sepharose 4B column) was added to the heated globulin fraction to make up for approximately 50% of the activity in unheated albumin fraction (about 0.1% of the diet). Other dietary ingredients were added in the proportions shown in Table 1. Carbohydrate, a 3:1 mixture of corn starch and sucrose, was used to adjust 100%. A protein-free diet was also prepared in which protein (10 g/100 g diet) was replaced by the carbohydrate mixture.

**Animals and feeding:** Male Wistar rats (7 rats/treatment) with an average weight of 52.3 ± 0.4 g were used. The rats were specific pathogen free (SPF) and were furnished by the University of Campinas (UNICAMP) Experimental Animal Center. They were kept individually in metabolic cages for 8 d for a nitrogen balance experiment in which each rat was allowed free access to diet and water. Before access to experimental diets, the rats had been fasted for 16 h and were housed in an air-conditioned room (21 ± 2°C) with a 12 h dark-light cycle. The 8-d experiment was divided into two periods: the first 3 d were time for adaptation to the diet and to the experimental environment; in the last 5 d, food intake was recorded and feces and urine collected for nitrogen determination. Body weight gain was recorded every other day. The experimental design was approved by the committee for Animal
Care at the Instituto de Tecnologia de Alimentos.

The following indices were calculated from the experimental data: dietary efficiency ratio (DER), defined as body weight gain/diet consumed; nitrogen retention (NR), defined as a difference between dietary nitrogen (NI) and fecal and urinary nitrogen; true digestibility (D), which is the ratio of nitrogen absorbed (NA) divided by the nitrogen intake; and true biological value (BV), the ratio of nitrogen retained (NR)/nitrogen absorbed (NA). The true indices in contrast to the apparent values (not shown) were obtained as the difference between experimental groups and the protein-free groups in fecal and urinary nitrogen.

**Statistical treatment.** A specific computer software program (ESTAT) was used. The experimental design was calculated by ANOVA, followed by Tukey’s test; the differences among means were considered significant at $p \leq 0.05$.

**RESULTS**

**Trypsin inhibitor and phytohemagglutinin (lectin) activities**

Protein content and trypsin inhibitor and phytohemagglutinin activities for the various bean fractions are presented in Table 2. The total content after dialysis and freeze-drying in the acidic extract was lower than that obtained by alkaline extraction. The insoluble residue was discarded, although it contained 16% protein, in a dry state. Bean protein was fractionated into globulin and albumin, their ratio in the initial extract being estimated at 2.

The trypsin inhibitor and phytohemagglutinin activities were both much higher in the albumin fraction than in the total protein concentrate and globulin fractions. Some activity passed into the insoluble residue.

Table 3 shows the results of determination of trypsin inhibitor and phytohemagglutinin activities in the diets containing bean protein fractions without heat treatment at 100°C for 30 min. Boiling the bean protein fractions in water for 30 min caused a complete loss of the phytohemagglutinin activity, but not of the

| Protein fraction          | Protein concentration (% dry basis) | Inhibitor activity$^a$ (UTI/mg P) | Hemagglutinin titer$^b$ (HT/mg P) |
|--------------------------|------------------------------------|-----------------------------------|----------------------------------|
| Total protein concentrate| 50.79 ± 0.03                       | 321                               | 157                              |
| Albumin fraction         | 38.00 ± 0.11                       | 728                               | 480                              |
| Globulin fraction        | 79.08 ± 2.06                       | 151                               | 148                              |
| Insoluble residue        | 16.00 ± 0.01                       | 34                                | 46                               |

$^a$ Units of trypsin inhibitor per milligram of protein.

$^b$ Hemagglutinin titer (HT) per milligram of protein.
Table 3. Relative trypsin inhibitor (UTI) and hemagglutinin activity (HT) per 100 g of diet prepared with the addition of heat-treated and untreated bean protein and casein.

| Dietary protein source | Dietary protein (g/100 g) | Inhibitor activity (UTI/100 g × 10⁻³) | Hemagglutinin activity (HT/100 g × 10⁻³) |
|------------------------|---------------------------|--------------------------------------|----------------------------------------|
| Casein                 | 10.26                     | (—)                                  | (—)                                    |
| Casein + unheated total protein concentrate a | 10.73                     | 250.0                                | 438.4                                  |
| Casein + heated total protein concentrate b | 10.08                     | 310.0                                | 0.0                                    |
| Casein + unheated albumin a | 10.85                     | 1,096.0                              | 1,517.0                                |
| Casein + heated albumin b | 10.66                     | 1,013.0                              | 0.0                                    |
| Casein + unheated globulin a | 10.24                     | 105.4                                | 660.5                                  |
| Casein + heated globulin b | 10.10                     | 102.2                                | 0.0                                    |
| Casein + heated globulin b + unheated inhibitor c | 10.05                     | 504.2                                | 0.0                                    |

a Diets with unheated bean protein contained 6% casein and 4% bean protein.
b The diet with heated bean protein contained 5% casein and 5% bean protein.
c Unheated isolate trypsin inhibitor was added to account for 50% of the albumin fraction activity.

trypsin inhibitor activity.

Nutritional and toxicological properties of bean trypsin inhibitor and phytohemagglutinin

The presence of unheated bean protein fraction in the diet, which contained both activities of trypsin inhibitor and phytohemagglutinin, drastically reduced diet intake and body weight gain. The diet efficiency ratio (body weight gain/diet intake) were much lower or negative in the groups fed unheated bean protein. On the other hand, the groups fed heated bean protein not having phytohemagglutinin activity showed normal diet consumption, body weight gain, and diet efficiency ratio, which did not differ from the casein diet (Table 4). Data on rats fed unheated albumin fraction were omitted because all of them died before the end of the experiment, revealing a very high toxicity of this protein fraction. The degree of inhibition of growth by bean protein intake was albumin > total protein concentrate > globulin, in that order.

Table 5 presents data on the nitrogen balance in rats fed various protein fractions. Nitrogen intake was proportional to body weight gain; thus it was highest in the casein-fed group and subsequently in the groups fed heated bean protein fractions with no statistical difference. On the other hand, fecal nitrogen was statistically higher in the groups receiving unheated bean protein fractions than in those receiving heated bean protein fractions. No statistical differences
Table 4. Diet consumption, body weight change, and diet efficiency ratio for rats fed diets containing heated and unheated bean fractions.1

| Protein sources                        | Diet consumption (g/rat/5 d) | Body weight change (g) | Diet efficiency ratio |
|----------------------------------------|------------------------------|------------------------|-----------------------|
| Casein                                 | 59.3 ± 4.4a                  | 23.4 ± 3.0a (100.0)²   | 0.39                  |
| Casein + unheated total protein concentrate | 21.5 ± 2.8c                | -0.2 ± 0.4c (—)        | 0.00                  |
| Casein + heated total protein concentrate | 49.7 ± 5.1a                  | 18.5 ± 2.2ab (79.0)    | 0.37                  |
| Casein + heated albumin*               | 40.5 ± 5.1a                  | 13.0 ± 2.1b (56.0)     | 0.32                  |
| Casein + unheated globulin             | 31.2 ± 7.5bc                 | 3.9 ± 3.1c (21.7)      | 0.12                  |
| Casein + heated globulin               | 55.7 ± 7.0a                  | 20.9 ± 3.6a (89.0)     | 0.37                  |
| Casein + heated globulin + unheated trypsin inhibitor | 56.7 ± 5.8a                | 17.6 ± 2.2ab (75.0)    | 0.31                  |
| Nonprotein diet                        | 27.6 ± 2.4c                  | -3.2 ± 0.5c (—)        | -0.12                 |

1 Results are mean ± SD of 7 rats in each diet.
2 Values in parentheses are % relative to casein.
* Diet containing unheated albumin was discarded because of the death of all rats before the end of the 5 d nitrogen balance.

*a, b, c Different superscript letters (columns) indicate statistically different results (p < 0.05).

were found in urinary nitrogen among groups fed heated and unheated bean protein fractions. Nitrogen retention tended to be higher in the casein-fed group, but was not statistically different from those in the groups fed heated bean protein fractions. The rats fed unheated bean protein fractions were inferior in nitrogen retention to those receiving casein or heated bean protein fractions (p ≤ 0.05). It is interesting to note that rats on the diet containing heated globulin fraction to which a purified unheated bean trypsin-chymotrypsin inhibitor was added behaved similarly to those fed a casein diet (Table 5).

Table 6 shows calculated data on protein quality indices from nitrogen balance.

Casein was the highest in digestibility, followed by heated bean protein fractions and heated globulin supplemented with purified trypsin inhibitor. Conversely, unheated bean protein fractions were significantly inferior in digestibility to heated bean protein fractions. In respect of biological value, heated bean protein fractions were not significantly different from casein, but unheated total bean protein concentrate was significantly inferior to casein (p ≤ 0.05).

NPU was higher for casein but did not significantly differ from heated total protein concentrate and heated globulin fractions with and without the addition of trypsin inhibitor (p ≤ 0.05). Nevertheless, heated albumin fraction was significantly different in digestibility from the other heated bean protein fractions. Unheated globulin fraction showed the lowest protein digestibility.
Table 5. Nitrogen balance (5 d) in rats fed diets containing heated and unheated bean fractions.1

| Protein sources                                | Nitrogen intake (mg) | Fecal nitrogen (mg) | Urinary nitrogen (mg) | Retained nitrogen (mg) |
|------------------------------------------------|----------------------|---------------------|-----------------------|------------------------|
| Casein                                         | 972.7 ± 71.8a        | 52.0 ± 10.0d        | 155.6 ± 60.4a         | 789.0 ± 12.6a          |
| Casein + unheated bean protein conc.            | 370.0 ± 47.8d        | 202.0 ± 27.7a       | 88.7 ± 25.3a          | 103.1 ± 50.7d          |
| Casein + heated bean protein conc.              | 800.8 ± 82.2ab       | 141.1 ± 17.8bc      | 87.3 ± 20.7a          | 596.2 ± 90.4bc         |
| Casein + unheated albumin*                      | 691.2 ± 128.9bc      | 117.6 ± 35.1c       | 146.3 ± 78.7a         | 451.2 ± 71.2c          |
| Casein + unheated globulin                      | 512.0 ± 122.6ad      | 208.8 ± 47.4a       | 100.5 ± 33.9a         | 226.6 ± 50.1d          |
| Casein + heated globulin                        | 901.3 ± 112.5ab      | 181.7 ± 16.1ab      | 108.3 ± 40.2a         | 635.1 ± 101.9ab        |
| Casein + heated globulin + unheated trypsin inhibitor | 1,018.4 ± 112.5a     | 173.1 ± 28.2abc     | 124.8 ± 25.2a         | 744.4 ± 128.3ab        |
| Nonprotein diet                                 | (−)                  | 12.9 ± 2.2d         | 10.3 ± 2.4b           | (−)                    |

1 Results are mean ± SD of 7 rats in each diet.
* Diet containing unheated albumin was not taken into consideration because all rats died before the end of the 5-d feeding.
abc Different letters superscript (columns) indicate statistically different results (p ≤ 0.05).

DISCUSSION

To minimize the toxic effect of unheated bean protein fractions, the diets were formulated with a mixture of casein and bean protein, thus preventing the rats from dying during the feeding period. Nevertheless, 4% of the unheated albumin fraction was sufficient to kill all the rats before ending the feeding period; therefore the data are not presented in this paper.

The acidic conditions used for extraction are not the ideal condition for quantitative extraction of Phaseolus vulgaris seed proteins; however, previous experiments (13) have shown that under these conditions, trypsin-chymotrypsin inhibitors and phytohemagglutinins are extracted more selectively to the detriment of other proteins.

Higher trypsin-chymotrypsin inhibitor activity in the albumin, comparative with other bean seed protein fractions, has been consistently shown (5, 11, 19).

Methionine and cysteine were added to the diets to make the protein mixture equal to or better than casein regarding amino acid composition. It has been demonstrated by various authors (20–22) that bean protein may have its nutritive
Table 6. True digestibility (D), biological value (BV), and net protein utilization of rats fed diets (5-d period) containing unheated and heated bean protein.\(^1\)

| Protein sources                        | Digestibility (D) (%) | Biological value (BV) (%) | Net protein utilization (NPU) (%) |
|----------------------------------------|-----------------------|---------------------------|----------------------------------|
| Casein                                 | 96.0 ± 1.0\(^a\)      | 84.8 ± 5.2\(^a\)          | 81.4 ± 5.0\(^a\)                 |
| Casein + unheated total protein concentrate | 52.1 ± 3.8\(^d\)     | 54.3 ± 10.0\(^b\)        | 26.7 ± 11.0\(^d\)                |
| Casein + heated total protein concentrate | 83.9 ± 2.7\(^b\)     | 88.4 ± 3.7\(^a\)        | 74.2 ± 11.9\(^ab\)               |
| Casein + heated albumin\(^*\)         | 85.7 ± 5.6\(^b\)     | 75.9 ± 9.7\(^a\)        | 65.3 ± 9.0\(^b\)                 |
| Casein + unheated globulin             | 61.4 ± 4.4\(^c\)     | 72.7 ± 6.3\(^ab\)       | 44.5 ± 2.5\(^c\)                 |
| Casein + heated globulin               | 81.1 ± 2.2\(^b\)     | 88.5 ± 5.1\(^a\)        | 70.3 ± 4.8\(^ab\)                |
| Casein + heated globulin + unheated trypsin inhibitor | 84.6 ± 3.2\(^b\)     | 86.4 ± 4.2\(^a\)        | 72.9 ± 5.9\(^ab\)                |

\(^{1}\) Results are mean ± SD of 7 rats in each diet for 5d.

\(^{a,b,c,d}\) Different superscript letters (columns) indicate statistically different results (\(p \leq 0.05\)).

\(^*\) The diet containing unheated albumin was not considered because it caused the deaths of all animals before the end of the experimental period.

value diminished by (a) a limited amount of sulfur-containing amino acids; (b) low digestibility of bean protein compared with other vegetable proteins; (c) presence in the bean seeds of heat-labile and heat-stable substances, which act as anti-nutritional factors.

The nutritional and toxicological properties of the trypsin inhibitor and phytohemagglutinin will be discussed below.

As seen in Table 2, both trypsin inhibitor and phytohemagglutinin activities are much higher in the albumin fraction than in the globulin fraction. It has been shown \((13, 23, 24)\) that the trypsin inhibitory activity in *Phaseolus* beans is associated with Bowman-Birk-type inhibitors, which simultaneously inhibit trypsin and chymotrypsin and are very resistant to heat inactivation. Alternatively, it is highly possible to inactivate hemagglutinin in the bean extract by heat treatment without any great loss of trypsin inhibitor activity \((25)\). In this investigation the phytohemagglutinin activity was completely deprived in water suspension that contained various bean protein fractions by heating at 100°C for 30 min.

The heat resistance of trypsin inhibitor and phytohemagglutinin is illustrated in Table 3. Although heat treatment completely eliminated the phytohemagglutinin activity, the trypsin inhibitor was essentially not affected. The clear difference in heat resistance between these two antinutritional factors enabled us to comparatively examine these harmful actions on rat growth and survival.
Although the Bowman-Birk type of inhibitor inhibits both trypsin and chymotrypsin in vitro, its intake seems to have no effect on the growth or diet-efficiency ratio (Table 4). Neither the inhibitor remaining resistant to heat in the bean protein fraction nor the intact purified inhibitor added to the diet interfered with protein digestion or utilization (Tables 5 and 6). Similar results had previously been demonstrated by Sgarbieri et al (23).

The apparent innocuity of naturally occurring trypsin inhibitor in the diet may be explained by Lyman and Lepkovasky (25), Green and Lyman (26), and Liener (27), who have demonstrated that the secretion of pancreatic enzymes undergoes a feedback control depending on the concentration of active trypsin in the intestinal lumen. As trypsin inhibitor binds irreversibly to trypsin, the level of active trypsin decreases, which results in the stimulation of pancreatic enzyme secretion mediated by cholecystokinin.

According to several explanations, by Struthers et al (28), Gumbmann et al (29), Liener et al (30), and Spangler et al (31), the pancreatic hypersecretion imposed by trypsin inhibitor in the intestinal lumen may cause pancreas hypertrophy and pancreatic lesions when the stimulus is sustained for a long period. Furthermore, this may cause an increased fecal excretion of pancreatic proteins, thereby giving rise to a serious deficiency in sulphur-containing amino acids causing impaired growth. These phenomena were reported for rats fed diets with raw soybean flour that contains the Kunitz and the Bowman-Birk trypsin inhibitors.

Burns (32) observed that feeding raw soybean or trypsin inhibitor for long periods did not cause pancreas hypertrophy in all animal species. Neither caused hypertrophy in dogs, pigs, or hamsters. Pancreatic lesions were observed in Wistar rats, but not in mice and hamsters.

Liener and Kakade (6) observed a direct correlation between pancreas hypertrophy and the size of the animal species, establishing that pancreas hypertrophy will occur only in species where the size of the pancreas will exceed 0.3% of the animal body weight.

The results reported in this paper clearly show that the active phytohemagglutinin is responsible for decreased food intake, low diet efficiency ratio, body weight loss, and poor protein utilization of rats fed diets containing mixtures of casein and unheated bean protein fractions (Tables 4–6).

Phytohemagglutinins occur in legume bean seeds in a concentration range of 2% to 10% of the total seed protein, and their presence during the whole life cycle of the plant suggests functions for these proteins besides those of a reserve protein (33).

Various physiological detrimental effects have been attributed to the presence of active phytohemagglutinin in the diet, such as retardation of growth (8), decrease in protein digestibility and nitrogen absorption (5), decrease in carbohydrate digestibility (34), alteration in the activities of intestinal tissues and liver enzymes (35), and decrease of blood insulin level (36).

It has been demonstrated that *Phaseolus* phytohemagglutinin is extremely
resistant to proteolysis in the gastrointestinal tract of rats (37). On ingestion, active phytohemagglutinin binds to microvilli of the small intestine enterocytes, which leads to extensive and severe damage to the proximal small intestine brush border of all monogastric animals studied so far (38, 39).

The physiological and nutritional consequences of phytohemagglutinin binding to small intestine enterocytes have been demonstrated at intestinal and systemic levels. At intestinal levels, the main observed effects have been (a) degradation of microvilli (38); (b) changes in intestinal permeability (35); (c) cell absorption of intact active phytohemagglutinin (40); and (d) faster renewal of intestinal cells and cell hypertrophy (41).

A growing body of evidence indicates that certain phytohemagglutinins are preferentially absorbed from the intestinal lumen. Up to 10% of kidney bean “cultivar processor” phytohemagglutinin can be absorbed intact when given by stomach intubation (42).

According to Pusztai et al (36), the underlying reasons for these effects on metabolism may be a result of the modulation of hormonal balance in the body. The blood insulin concentration of rats fed native kidney beans or soybean proteins was depressed. Changes in general metabolism because of a modulation of endocrine hormonal control may occur either through a direct effect on tissue cells of the systemically absorbed phytohemagglutinin or indirectly through an interaction of the dietary phytohemagglutinin with intestinal endocrine cells. This may affect the hormone output of systemic endocrine cells.

The data presented and discussed in this paper, both experimental and from the literature, allow a conclusion that the main toxic and antinutritional factor in Phaseolus vulgaris bean is phytohemagglutinin.

Phytohemagglutinin can be heat inactivated under conditions in which trypsin-chymotrypsin inhibitor remains totally active. Therefore the antinutritional and toxicity action of both types of protein could comparatively be evaluated. Phytohemagglutinin drastically impaired growth and dietary protein utilization, probably by damaging the intestinal epithelium and interfering with body hormonal balance, for example, the levels of circulating insulin and thyroid hormones.

Bowman-Birk trypsin inhibitor did not cause growth depression or interfere with protein utilization, at least in the short period of this experimental study.

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