

The Effect of Feeding Tepary Bean (*Phaseolus acutifolius*) Proteinase Inhibitors on the Growth and Pancreas of Young Mice^{1,2}

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Abstract: The effect of tepary proteinase inhibitor (TI) isolated from unheated tepary bean on the growth, nitrogen digestion and pancreatic hypertrophy of mice was assessed. The mice were fed a whole protein based diet containing either 0, 100, 200, 400 or 600 mg TI per 100 g of diet. Growth performance of mice fed whole egg or 100 mg TI/100 g diet was superior to that of mice fed 200, 400 and 600 TI mg/100 g diet. The growth inhibition of mice fed trypsin inhibitor above 200 mg TI/100 g diet was accompanied by a lower apparent digestibility of protein and lower food conversion efficiency. The pancreas weight relative to body weight of animals fed TI was significantly higher than that of animals fed whole egg. The results of the present study also indicate that TI is not responsible for the death of mice and rats fed raw tepary bean flour.

Key words: Tepary bean, *Phaseolus acutifolius*, proteinase inhibitors

Introduction

Tepary bean (*Phaseolus acutifolius*) is a drought resistant desert legume of southwestern North America, where it has been used in Native American diets for centuries. As in other legumes, the main nutritional advantage of the tepary bean lies in the mature seeds which contain 21-31.9% protein, 0.9-1.17% fat and 65.3-69.1% carbohydrates (Scheerens *et al.*, 1983). Currently tepary bean seeds are rarely consumed and their high protein and carbohydrate content are under utilized. However, like other legumes, tepary bean contains trypsin inhibitor and hemagglutinin (lectin).

Tepary bean was reported to be very toxic in the raw state causing great weight loss, negative protein efficiency ratio (PER), negative net protein utilization (NPU), poor protein digestibility, and the death of rats and mice within 10 days (Gonzales-Garza *et al.*, 1982; Grant *et al.*, 1983; Scheerens *et al.*, 1983). Intraperitoneal injection of crude extract from tepary bean was also toxic, while autoclaving resulted in destruction of the intraperitoneal toxicity of the extract (de Muelenaere, 1964). Sotelo *et al.* (1983) reported that intra gastric administration of a saline extract of tepary beans to rats caused widespread destruction of the microvilli of absorptive intestinal cells and disruption of the endoplasmic reticulum profile. These authors attributed the toxicity and poor nutritive value of tepary bean to high concentrations of phytohemagglutinin. Soaking and cooking of tepary bean was found to

increase PER and support animal growth (Gonzales de Mejia *et al.*, 1988; Idouraine *et al.*, 1992). Based on heat treatment data, Idouraine *et al.* (1992) suggested that trypsin inhibition, rather than hemagglutinin, might be the major cause of the toxicity.

This controversy as to which factor in tepary bean is responsible for certain anti-nutritional or physiological effects in experimental animals seems to have arisen because most investigations were carried out using crude extract or raw tepary bean flour, but not pure inhibitor isolated from the tepary bean. Therefore the objectives of this study were:

- 1 to evaluate the growth, nutrient digestibility and PER responses of mice to diets containing 100, 200, 400 and 600 mg/100 g tepary bean proteinase inhibitor;
- 2 to determine whether incorporation of proteinase inhibitor from tepary bean into the diet would produce pancreatic hypertrophy in mice.

Materials and Methods

Trypsin inhibitor purification: Tepary bean proteinase inhibitor was purified from tepary flour as described by Osman and Weber (1994) using a process of extraction with Tris-HCL, pH 7.0 containing 0.5 M NaCl, heat treatment, ammonium sulfate precipitation, dialysis deionized distilled water (dd H₂O) and ultrafiltration to concentrate and remove low molecular weight components. The final purification was achieved by chromatography on DEAE ion exchange column.

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Table 1: Composition of experimental diets (g/100 g)^a

Ingredient	Tepary bean proteinase inhibitor (mg/100 g)				
	Control	100	200	400	600
Whole egg	17.39	17.39	17.39	17.39	17.39
Tepary inhibitor (mg)	0.0	100.0	200.0	400.0	600.0
Cerelose	60.45	60.35	60.25	60.05	59.85
Corn oil	3.00	3.00	3.00	3.00	3.00
Cellulose	3.00	3.00	3.00	3.00	3.00
AIN-76 vitamin mix ¹	1.00	1.00	1.00	1.00	1.00
AIN-76 mineral mix ²	3.50	3.50	3.50	3.50	3.50
Chromium oxide	0.20	0.20	0.20	0.20	0.20
Choline chloride	0.20	0.20	0.20	0.20	0.20
Butylated hydroxy toluene	0.002	0.002	0.002	0.002	0.002
DL-Methionine	0.05	0.05	0.05	0.05	0.05
Bentonite	11.208	11.208	11.208	11.208	11.208
Total	100.00	100.00	100.00	100.00	100.00

^aProtein = 8.1%; egg (metabolizable energy) = 12.89 kJ/g (3.08 kcal/g). ¹Composition (per kg mixture): thiamin hydrochloride, 600.0 mg; riboflavin, 600.0 mg; nicotinic acid, 3.0 g; pyridoxine hydrochloride, 700 mg; D-biotin, 20.0 mg; cyanocobalamin (vitamin B₁₂), 1.0 mg; retinal palmitate (vitamin A), 800 mg; DL-alpha-tocopheryl acetate (pre-mix), 20.0 g; cholecalciferol (vitamin D₃), 2.5 mg; menaquinone (vitamin K), 5.0 mg; sucrose (finely powdered), 972.9 g. ²Composition (per kg mixture): calcium phosphate (dibasic), 500 g; sodium chloride, 74 g; potassium citrate (monohydrate), 220 g; potassium sulfate, 52 g; magnesium oxide, 24 g; manganous carbonate, 3.5 g; ferric citrate (16-18% Fe), 6 g; zinc carbonate, 1.6 g; cupric carbonate, 0.3 g; potassium iodate, 0.01 g; sodium selenite (1.5 H₂O), 0.0075 g; chromium potassium sulfate, 0.55 g; sucrose (finely powdered), 118 g.

Diets: A whole egg basal diet was prepared to provide about 8% protein (Cossack and Weber, 1983) and five experimental diets were prepared by adding 0, 100, 200, 400 or 600 mg of purified tepary bean proteinase inhibitor per 100 g of basal diet (Table 1).

Animals: The feeding study was conducted with three week-old weanling male mice of the Charles River CD-1 strain and was approved by The University of Arizona Institutional Animal Care and Use committee. The mice were randomly separated into 5 groups of 10 mice each, which were housed individually in suspended stainless steel cages in The University of Arizona AAALAC accredited animal care facility. The room temperature was maintained at 22 °C with a 12 h light:dark. Feed and water were provided ad libitum for three weeks. Body weight and feed consumption were recorded twice weekly. Protein efficiency ratio was calculated as described by the Association of Official Analytical Chemists (AOAC, 1990a). Feces were also collected twice a week, separated from wasted feed, and stored at -70 °C. At the end of the experiment the fecal samples were dried in vacuum oven at 70 °C and ground to pass through a 40-mesh screen and used to determine protein content and the apparent protein digestibility. At the end of 21 days the animals were anesthetized with CO₂ and decapitated. The pancreas was excised, weighed and frozen at -70 °C.

Analyses: Crude Protein Determination of Diets and

Feces. Protein content (N × 6.25) of the diet and feces were determined by the standard micro kjeldahl procedure (AOAC, 1990b). Samples were analyzed in triplicate.

Chromium Oxide Determination of Diets and Feces. The apparent digestibility was estimated in triplicate dry samples according to the method of Schürch *et al.* (1950).

Statistical Analysis: The data were analyzed using one-way ANOVA with means separated by least significance difference (LSD) at P<0.05 (Steel and Torrie, 1960).

Results and Discussion

Growth: Weight gain of mice was affected by the level of tepary inhibitors in the diet. Body weights of mice fed 200, 400 and 600 mg TI/100 g diet were significantly lower (P<0.05) than those of mice fed either the basal diet or a diet containing 100 mg TI/100 g diet, but there were no body weight differences (P>0.05) among mice fed 200, 400 or 600 mg TI/100 g diet. These results agree with those reported by Roy and Schneeman (1981) who also observed no significant differences in body weight gain among three groups of animals fed high levels of soybean trypsin inhibitor. Similarly Gertler and his group (Birk and Gertler, 1961; Gertler *et al.*, 1967; Gertler and Nitsan, 1970) demonstrated that addition of Bowman-Birk or Kunitz inhibitors to heated soybean meal depressed growth rate and reduced metabolized energy in rats and chicks. Rackis (1965)

Table 2: Food intake, growth rate, PER, N-digestibility and pancreatic weight in mice fed tepary inhibitor

Diet	Protein Source	Tepary Inhibitor mg/100	Food Intake g, mice/day	Growth Rate g mice/day	PER	N-digestibility	Pancreas wt g/100 g body wt
1	Whole egg tepary inhibitor	0.0	4.61 ± 0.29 ^a	0.87 ± 0.18 ^a	2.35 ± 0.42 ^a	88.12	0.65 ± 0.13 ^a
2	Whole egg tepary inhibitor	100	4.80 ± 0.58 ^a	0.76 ± 0.17 ^a	2.01 ± 0.37 ^a	84.01	0.71 ± 0.11 ^a
3	Whole egg tepary inhibitor	200	4.80 ± 0.42 ^a	0.65 ± 0.15 ^b	1.68 ± 0.44 ^b	83.16	1.11 ± 0.11 ^b
4	Whole egg tepary inhibitor	400	4.92 ± 0.47 ^a	0.57 ± 0.15 ^b	1.45 ± 0.64 ^b	80.26	1.23 ± 0.25 ^b
5	Whole egg tepary inhibitor	600	5.13 ± 0.44 ^a	0.54 ± 0.07 ^b	1.32 ± 0.23 ^b	78.44	1.25 ± 0.18 ^b

^{a,b}Mean values with the same superscript within each column are not significantly different ($P > 0.05$).

also showed that the addition of 0.45 and 0.63% of purified Kunitz inhibitor to a casein diet reduced weight gain and lowered protein efficiency, but not to the same extent as raw soybean with the same level of trypsin inhibitor activity.

Protein efficiency ratio was also affected by the level of TI in the diets in the present study. No difference ($P > 0.05$) was observed in the PER of mice fed the whole egg basal diet (2.35) and mice fed 100 mg TI/100 g diet (2.01). However, as the level of tepary inhibitor increased (200, 400 and 600 mg) PER decreased significantly (1.68, 1.45 and 1.32 respectively), compared to the PER of whole egg. Gumbmann *et al.* (1989) also showed that feeding mice 200 mg soybean trypsin inhibitor/100 g casein diet significantly depressed the nutritional quality of the diet (PER) and body weight. Similar PER values for whole egg diets have been reported by Cossack and Weber (1983); Jensen and Weber (1987); Idouraine *et al.* (1992).

Food Consumption: There were no significant differences in the average food consumption among dietary groups (Table 2), indicating that TI did not interfere with the food intake of the mice. Similarly, Roy and Schneeman (1981) reported that the addition of 100 mg soybean TI/100 g casein diet did not affect food consumption in mice. This is in contrast to the response of chicks and rats, in which food consumption was significantly reduced when the level of soybean trypsin inhibitor in the diet increased (Gertler *et al.*, 1967). This may be due to the fact that pure trypsin inhibitors were added to heated soybean flour which may contain other antinutritional factors such as tannin. Tannin is known to form complexes with protein and minerals leading to decreased bioavailability of these nutrients.

Nitrogen Digestibility: The apparent nitrogen digestibility of young male mice fed diets containing TI showed that nitrogen digestibility is remarkably decreased as the amounts of TI in the diets increase. At 400 and 600 mg TI/100 g diet the apparent digestibility was significantly

reduced to 80.3 and 78.4%, respectively, when compared to 88.1% for basal diet. These results were in agreement with data from rats, chicks and guinea pigs, which demonstrated that the addition of soybean trypsin inhibitor to casein or heated soybean flour lowered the apparent nitrogen digestibility (Garlich and Nesheim, 1966; Peace *et al.*, 1992). The gradual decrease in nitrogen digestibility as the TI level increased in the diet, is likely due to lowered trypsin and chymotrypsin activities in the intestine by TI.

Pancreatic Hypertrophy: In general, pancreatic weight relative to body weight increased when the level of tepary inhibitor increased in the diet (Table 2). Significant ($P < 0.05$) pancreatic hypertrophy occurred in mice fed 200, 400 and 600 mg TI mg/100 g diet when compared to mice fed whole egg diet. There was no significant differences observed in mice fed whole egg and mice fed 100 mg TI/100 g diet. These results clearly demonstrate that young male mice were very sensitive to TI. The stimulation of pancreatic hypertrophy by feeding soybean trypsin inhibitor to mice, rats and chicks has been reported by research groups (Rackis, 1965; Gertler and Nitsan, 1970; Roy and Schneeman, 1981; Gumbmann *et al.*, 1989). The mechanism by which growth depression and pancreatic hypertrophy occur is best explained by the loss of inhibitor-bound pancreatic enzyme through excretion in the feces, and the compensatory synthesis and hypersecretion of such enzymes brought about by trypsin inhibitor ingestion (Lyman and Lepkousky, 1957; Rackis and Gumbmann, 1981; Liener, 1995). Hypersecretion results in pancreatic hypertrophy and nutritional stress due to the loss of essential amino acids, particularly methionine and cystine which are present in pancreatic proteinase in relatively high amounts.

No mortality occurred in mice fed diets with added TI, during the three-week experiment, indicating that TI was not responsible for the death of mice fed raw tepary bean flour. These results agree with Idouraine *et al.* (1992) who suggested the presence of a toxic factor that was responsible for the death of experimental animals

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fed raw tepary bean flour. Gonzales-Garza *et al.* (1982) found that the tepary bean protein fraction devoid of trypsin and hemagglutinin activities exhibited a cytotoxic activity on rat intestinal epithelial cells similar to that of hemagglutinin and trypsin inhibitor fractions. Similarly, Schingoethe *et al.* (1974) reported separation of low molecular weight (5,000 Daltons) compounds from soybean that reduced the growth rate and feed efficiency of mice without causing pancreatic enlargement. Hamaguchi *et al.* (1977) isolated a lethal protein with hemagglutinating activity, but not trypsin inhibitor activity, from kintoki bean (*Phaseolus vulgaris*) that induced acute toxicity and caused the death of all mice given 250 Fg/g body weight interperitoneal injections of the protein. Because tepary hemagglutinin is completely destroyed by heat treatment, the high mortality rate of mice and rats fed autoclaved tepary bean might be due to the presence of an unknown heat stable toxic factor.

In conclusion, this study has demonstrated that feeding a diet containing TI to weanling mice for 21 days resulted in significantly reduced growth rate, PER and apparent protein digestibility, but not reduced food consumption when compared with mice fed a control diet containing whole egg. All the diets contained whole egg as the sole source of dietary protein at a level of 8%. Addition of TI to the whole egg diet also caused pancreatic hypertrophy. Results of this study also demonstrated that TI was not primarily responsible for the toxicity of raw tepary bean.

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