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# Nutritional study of two Brazilian soybean (*Glycine max*) cultivars differing in the contents of antinutritional and toxic proteins

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**Abstract**

The research was conducted with two different recently released Brazilian soybean cultivars (Rio Balsas and Bays) to evaluate whether there is any correlation between the different levels of antinutritional and/or toxic proteins in the cultivars and their nutritive value as sources of protein for monogastric animals (rats). Furthermore, it is discussed, for the first time, the role of the dietary soyatoxin on the performance of rats fed on diets containing soyatoxin-rich (cv. Bays) and soyatoxin-free (cv. Rio Balsas) soybean cultivars. Feeding rats with diets containing raw soybean cultivars showed a lower growth rate, net protein utilization and digestibility, a much higher dry matter and nitrogen excretion and macroscopic alterations in internal organs when compared to rats fed on egg-white protein. The nutritional parameters measured for the diet based on raw Bays cultivar were poorer than those of the diet prepared with Rio Balsas. In the raw soybeans, trypsin inhibitor and lectin, and urease to a lesser extent, significantly affected at different fashion the soybean protein utilization. Heating treatment of the Bays seeds increased the growth rate, NPU, in vivo protein digestibility and practically eliminated or attenuated all the organ alterations observed. This study might be helpful in the choice of safe and nutritious soybean cultivars. 

*Keywords:* *Glycine max*; Soybean; Toxicity; Protein quality; Seed protein

## 1. Introduction

Seed legumes provide one-fifth of all plant proteins consumed by man on a global basis [1,2]. However, some legumes, in particular soybeans, contain significant amounts of bioactive or antinutritional components that can possibly alter the body metabolism of consumers [3,4,5]. The major proteins responsible for the low nutritional value of raw soybean meals are trypsin inhibitors and lectin, however, it has been suggested that other natural compounds may also contribute to the deleterious effects observed [6,7]. Vasconcelos et al. [8] showed that soybeans sold for consumption in Rio de Janeiro (Brazil) contain a protein, named soyatoxin, which is severely toxic to mice and rats when intraperitoneally injected.

Heating treatments have been used to improve the nutritional quality of soybeans [3,9,10], but they should be kept to a minimum due to the cost and the possibility of destroying important amino acids [11] and reducing other nutrient availability [12,13]. Another alternative to abolish or diminish the detrimental effects of these constituents is the use of genetic and/or molecular approaches, developing plants with low levels, or even totally free of these substances. In fact, recently developed soybean isolines, deficient in Kunitz protease inhibitor or lectin, showed improved nutritional quality [11,14].

Brazilian soybean improvement program has employed

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breeding strategies for developing cultivars adapted to low latitudes to supply new regions with this crop and hence, increase production. However, there is a lack of adequate information on their nutritional potentials. Recently, Vasconcelos et al. [15] reported on the composition and the presence of antinutritional and/or toxic factors in new Brazilian soybean cultivars developed by EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária). They suggested that Rio Balsas cultivar should be one choice for breading programs since it shows a high protein content, is soyatoxin-free and contains relatively low levels of trypsin inhibitors, lectin and urease. This study was designed to establish what relationship, if any, might exist between the biological parameters and the antinutritional and/or toxic factors present in two Brazilian soybean cultivars. Furthermore, it is discussed, for the first time, the role of the dietary soyatoxin on the performance of rats fed on diets containing soyatoxin-rich (cv. Bays) and soyatoxin-free (cv. Rio Balsas) cultivars.

## 2. Methods and materials

### *2.1. Materials*

Seeds of soybean [*Glycine max* (L.) Merr.], Rio Balsas and Bays cultivars, adapted to Brazilian low latitudes were developed and supplied by EMBRAPA (Piauí, Brazil). Casein was purchased from Merck (Darmstadt, Alemanha). Egg white, soybean trypsin inhibitor, type I-S, and urease, 41H7008, from Sigma Chemical Co. (St. Louis, USA). All the other chemicals used were of analytical grade.

### *2.2. Antinutritional and/or toxic proteins*

Crude extracts were prepared according to Vasconcelos et al. [15] and used for detection of the hemagglutinating, toxic and urease activities. The protease inhibitor assay was carried out by a slight modification of the method originally described by Kakade [16]. Hemagglutinating activity was assayed according to Vasconcelos et al. [17]. Toxic activity was defined as mortality observed in mice within 24 h after intraperitoneal (ip) injections of the crude extracts [8]. Urease assay was carried out by minor modifications of the procedure described by Kaplan [18].

### *2.3. Amino acid composition*

Defatted soybean flours were hydrolyzed with 6 M HCl containing 1% phenol for 24 h under nitrogen atmosphere. The amino acid compositions were established after chromatography on Biochrom 20 system (Pharmacia). Tryptophan was determined according to the method described by Pintér-Szakács and Molnár-Perl [19].

### *2.4. Diets*

Soybean samples used for preparation of the diets were ground in a coffee grinder. Cooked bean samples were prepared by soaking in distilled water (1:4, w/v) for 60 min and boiling at 92°C for 60 min. These conditions were sufficient to abolish the toxic, trypsin inhibitory, hemagglutinating and urease activities. Cooked seeds and residual cooked water were blended, freeze-dried and ground into meals. Diets were prepared to contain the equivalent of 100 g protein/kg diet (Table 1) in the form of casein, or egg-white protein (EW), or cv. Rio Balsas (raw) or Bays (raw and cooked). Diets containing raw seed meal were supplemented with L-tryptophan and L-methionine based on the amino acid contents of the raw seeds, to bring the amino acid content to the target requirements for rats [20]. A diet containing no protein (NPC) was fed to allow determination of some nutritional parameters.

### *2.5. Feeding trials*

Wistar male rats were weaned at 21 days of age and given a commercial stock diet until their weights reached 55–60 g. They were fed the casein diet *ad libitum* for 3 days as a period of adaptation to pulverized diets and were selected according to food consumption and body weight. The animals were divided into 5 groups of twelve rats each, housed individually in screen-bottomed cages and fed control (egg-white), non-protein containing (NPC) or experimental diets (raw or cooked soybean meal) for 10 days. Feed and water were supplied *ad libitum*. Rat weights, diet spillage and refused diet were recorded daily. Feces were collected during the last 5 days of the experimental period, bulked, freeze-dried, weighed and ground in a coffee grinder. At the end of the trial the rats were killed by ether overdose and the internal organs dissected. These were then freeze-dried while the carcasses were dried in a oven at 100°C for 24 h. Dry weights were recorded before incorporating the organs with their original carcasses which were then ground and kept in a desiccator for appropriate analyses.

### *2.6. Chemical analyses*

Diets, carcasses and ground fecal samples were analyzed for moisture content [21] and total nitrogen [22]. The data were used to calculate apparent protein digestibility and net protein utilization (NPU) based on the method described by Miller and Bender [23]. All the results were calculated for each rat and the mean calculated within a group.

### *2.7. Statistical analyses*

The results were subjected to a one-way analysis of variance and the significance between means determined by Student's *t* test, and Tuckey's honest test when comparing

Table 1
Composition (g/kg) of NPC, EW and experimental diets[a]

| Ingredients | Casein | NPC | EW | Rio Balsas | Bays | |
|---|---|---|---|---|---|---|
| | | | | | Raw | Heated |
| Maize starch | 377 | 500 | 380.2 | 245.9 | 235.6 | 227.9 |
| Potato starch | 100 | 100 | 100 | 100 | 100 | 100 |
| Glucose | 150 | 150 | 150 | 150 | 150 | 150 |
| Maize oil | 150 | 150 | 150 | 150 | 150 | 150 |
| Vitamin mix[b] | 50 | 50 | 50 | 50 | 50 | 50 |
| Mineral mix[b] | 50 | 50 | 50 | 50 | 50 | 50 |
| Casein | 123 | — | — | — | — | — |
| Egg white | — | — | 119.8 | — | — | — |
| Rio Balsas | — | — | — | 251.6 | — | — |
| Bays | | | | | | |
| Raw | — | — | — | — | 261.9 | — |
| Cooked | — | — | — | — | — | 272.1 |
| L-methionine[c] | — | — | — | 1.5 | 1.6 | — |
| L-tryptophan[c] | — | — | — | 1.0 | 0.9 | — |

[a] CAS, casein; NPC, non-protein control; EW, egg-white protein.

[b] Vitamin mix (g/kg): vitamin $B_{12}$ (100%), 0.02; folic acid, 0.04; biotin (1%), 4.0; pyridoxine HCl, 0.04; thiamine HCl, 0.06; riboflavin (99%), 0.21; Ca-pantothenato (45%), 1.2; nicotinic acid, 4.0; inositol, 4.0; *p*-amino-benzoic acid, 12.0; choline chloride (50%), 24.0; maize starch, 950.43. Mineral mix (g/kg): calcium citrate, 296.1; calcium carbonate (40%), 65.8; copper carbonate, 1.1; magnesium carbonate, 34.3; zinc carbonate, 0.48; ferric citrate, 9.1; magnesium chloride.$6H_2O$, 5.82; sodium chloride, 74.0; potassium chloride, 119.5; monobasic calcium phosphate, 108.2; dibasic potassium phosphate, 210.1; sodium fluoride, 0.48; potassium iodate 0.1; magnesium sulfate, 75.4.

[c] Diets containing raw seed meal of the distinct cultivars were supplemented with L-methionine and L-tryptophan according to their amino acid compositions.

multiple means. Multiple regression analysis was applied, relating trypsin inhibitor, lectin, soyatoxin and urease to feed intake, weight gain, NPU and digestibility. Due to the high degree of multicolinearity presented in the samples among the independent variables a technique of orthogonalization was called in [24]. Principal components were constructed and a special test [25] was done to discard non-significant components. Then, regressions of the nutritional parameters on the principal components retained were run. From these regressions the coefficients of the original independent variables were recovered [26]. Additionally, to discriminate among the magnitude of impacts of changes in the independent variables on the dependent ones tests of difference in the size of the coefficients were done ($\alpha = 0.05$).

## 3. Results

### 3.1. Antinutritional and/or toxic proteins

The soybean trypsin inhibitory, lectin, toxic and urease activities are depicted in Table 2. These data are in agreement with those previously reported [15]. Except for the lectin, the Bays cultivar presented trypsin inhibitor, urease and soyatoxin contents significantly higher than those of Rio Balsas. The trypsin inhibitory and urease activities determined in the crude extract from Bays cultivar were about two-fold higher than the activities found for Rio Balsas which is soyatoxin-free. Indeed, the crude extract from Rio Balsas cultivar was not lethal when injected ip, even using a dose (1.0 g/kg mouse body weight) almost eight times higher than the one used from Bays. The hemagglutinating activity measured against rabbit erythrocytes was not significantly different between the two cultivars.

Table 2
Trypsin inhibitory, lectin, toxic and urease activities[a] present in the crude extracts from Brazilian soybean cultivars

| Activities | Cultivar | |
|---|---|---|
| | Rio Balsas | Bays |
| Trypsin inhibitory[b] | 30.6 ± 1.1[a] | 62.5 ± 2.6[b] |
| Lectin[c] | 6.5 ± 1.1[a] | 6.4 ± 1.9[a] |
| Toxic[d] | NL[e] | 0.137 ± 0.020 |
| Urease[f] | 107,320 ± 9,470a | 219,280 ± 12,600[b] |

[a] Values in a horizontal row with different following letters differ significantly ($P < 0.05$). Each value is an average of triplicate determinations.

[b] Trypsin inhibitory activity is expressed as g of trypsin inhibited per kg of flour.

[c] Lectin activity is expressed as grams of lectin equivalents per kg defatted meal.

[d] Toxic activity is represented as $LD_{50}$, 50% lethal dose. One $LD_{50}$ designates the amount of protein in g/kg of mouse body weight producing convulsion and death of 50% of tested animals injected by intraperitoneal route.

[e] Not lethal even at a dose of 1.0 g per kg of mouse body weight.

[f] Urease activity is shown as units of enzyme per kg of flour. The units were calculated from Sigma information that 1 g of pure enzyme contains 870.000 units.

Table 3
Comparison of amino acid composition (g per 16 g of N) of raw and heated soybean flours with FAO/WHO/UNU [27] patterns of amino acid requirement for different age groups and with those required for rats [20]

| Amino acid | Rio Balsas | Bays | | Child | | Rats |
|---|---|---|---|---|---|---|
| | | Raw | Cooked | 2–5 years | 10–12 years | |
| Asx | 11.34 | 11.85 | 9.56 | | | |
| Thr | 3.81 | 3.76 | 4.86 | 3.4 | 2.8 | 4.00 |
| Ser | 4.23 | 4.50 | 5.50 | | | |
| Glx | 18.30 | 19.02 | 15.85 | | | |
| Pro | 5.33 | 5.31 | 6.00 | | | |
| Gly | 3.88 | 3.78 | 5.88 | | | |
| Ala | 4.25 | 4.10 | 6.54 | | | |
| Cys | 1.63 | 1.44 | 0.39 | 2.5[a] | 2.2[a] | 4.50[a] |
| Met | 1.38 | 1.50 | 0.59 | | | |
| Val | 4.66 | 4.60 | 5.60 | 3.5 | 2.5 | 5.50 |
| Ile | 3.79 | 3.75 | 5.23 | 2.8 | 2.8 | 5.00 |
| Leu | 7.68 | 7.63 | 8.10 | 6.6 | 4.4 | 8.00 |
| Tyr | 5.02 | 4.73 | 4.00 | 6.3[b] | 2.2[b] | 9.00[b] |
| Phe | 6.05 | 5.82 | 5.10 | | | |
| His | 3.04 | 2.90 | 2.95 | 1.9 | 1.9 | 2.50 |
| Lys | 6.55 | 6.63 | 6.40 | 5.8 | 4.4 | 6.00 |
| Arg | 8.52 | 7.97 | 6.98 | | | 5.00 |
| Trp | 0.54 | 0.69 | 0.47 | 1.1 | 0.9 | 1.50 |
| Total | 100.00 | 99.98 | 100.00 | | | |

[a] Cys + Met.
[b] Tyr + Phe.

### 3.2. Amino acid composition

Before preparing the diets containing the seed meals as protein source, it was determined the amino acid composition of the soybean meals (Table 3) in order to eliminate the effects of the deficiency of these constituents. Comparison of the essential amino acid levels with FAO/WHO/UNU [27] pattern of amino acid requirements for children (2–5 and 10–12 years) suggests that the meals of Rio Balsas and Bays cultivars contain higher levels of essential amino acids than those from the standard, except for tryptophan. However, the seed meals were deficient in tryptophan (first limiting) as well as methionine + cystein (second limiting), when compared to the requirements for rats [20]. Thus, the diets containing the raw seed meals were supplemented with tryptophan and methionine. The heating treatment of Bays cultivar diminished the sulfur amino acid and tryptophan contents in relation to the provisional requirements for rats.

### 3.3. Nutritional parameters

At the end of the experimental period, the body weights of rats fed on raw Rio Balsas or Bays diets were similarly much lower, approximately 30%, than those of rats fed on egg-white (Table 4). However, the group fed on Rio Balsas diet showed weight gain slightly higher than the rats submitted to Bays diet. The low growth rate probably might be, in part, associated to food intake since the rats fed on the

Table 4
Nutritional parameters of rats fed on Rio Balsas and Bays seed meals compared[a] with those of rats fed on EW and NPC diets

| | Diets[b] | | | | |
|---|---|---|---|---|---|
| | NPC | EW | Rio Balsas | Bays | |
| | | | | Raw | Cooked |
| Initial body weight[c] (g) | 66.8 ± 2.8[a] | 66.8 ± 2.9[a] | 66.3 ± 2.8[a] | 67.2 ± 1.7[a] | 67.1 ± 1.5[a] |
| Final body weight[c] (g) | 51.9 ± 2.0[e] | 107.0 ± 5.5[a] | 75.3 ± 5.0[c] | 73.6 ± 4.2[d] | 84.4 ± 4.9[b] |
| Daily food intake[c] (g) | 6.3 ± 0.2[d] | 11.7 ± 0.4[a] | 8.1 ± 1.6[c] | 7.4 ± 1.4[c,d] | 9.8 ± 0.3[b] |
| NPU[d] | — | 93.6 ± 0.9[a] | 40.6 ± 0.9[c] | 31.4 ± 1.1[d] | 69.5 ± 0.4[b] |
| Protein digestibility (%)[d] | — | 98.2 ± 0.3[a] | 59.7 ± 3.0[c] | 50.9 ± 1.0[d] | 78.3 ± 1.9[b] |
| Body nitrogen[c] (g/kg) | 91.0 ± 0.6[a] | 78.0 ± 0.6[a] | 79.0 ± 0.7[a] | 84.0 ± 0.9[a] | 84.0 ± 0.2[a] |

[a] Values in a horizontal row with different letters differ significantly ($P < 0.05$).
[b] For key to diets see material and methods.
[c] Per rat.
[d] Per group of 12 rats.

Table 5
Relative fecal dry matter and nitrogen outputs of rats[a] fed on control (EW) and experimental diets calculated for the last 5 days

| Diets | Diet intake (g per rat) | N intake (g per rat) | Fecal output (g per rat) | Fecal N (g per rat) | Fecal output (×100) / Diet intake | Fecal N (×100) / N intake |
|---|---|---|---|---|---|---|
| EW | 60.4 ± 1.6a | 1.07 ± 0.02a | 1.80 ± 0.04a | 0.12 ± 0.01a | 3.0 ± 0.1a | 11.2 ± 0.5a |
| Rio Balsas | 39.2 ± 2.1b | 0.71 ± 0.09b | 2.71 ± 0.51b | 0.28 ± 0.03b | 6.9 ± 0.3b | 39.4 ± 2.6b |
| Bays | | | | | | |
| Raw | 38.3 ± 2.4b | 0.67 ± 0.07b | 3.26 ± 0.19c | 0.36 ± 0.02c | 8.5 ± 0.6c | 63.2 ± 2.4c |
| Cooked | 47.1 ± 1.4c | 0.83 ± 0.02c | 2.19 ± 0.12d | 0.16 ± 0.01d | 4.6 ± 0.2d | 19.3 ± 1.1d |

[a] Values in a vertical row with different following letters differ significantly ($P < 0.05$).

seed protein-based diets ate much less than those on egg white control. Although the consumption of the two raw soybean diets was not statistically different to each other, there was a trend for loss of appetite in rats fed on Bays cultivar, which in turn did not show significant difference when compared to rats fed on NPC diet. The net protein utilization (31.4–40.6%) and *in vivo* protein digestibility (50.9–59.7%) values were markedly decreased in rats fed raw soybean meals compared to those calculated for egg white fed rats (93.6% and 98.2%, respectively). Comparison of the nutritional parameters for rats fed on Bays and Rio Balsas diets, revealed that, in general, the first was poorer than the second one, as shown by their NPU and protein digestibility values. Total food consumption, body weight gain, NPU and protein digestibility increased with heating treatment, showing that the presence of heat-labile antinutritional and/or toxic proteins may have a bearing on the adverse effects observed following ingestion of raw soybean meal.

Table 5 shows that raw soybean fed rats had relative fecal dry matter outputs superior to those of egg-white fed rats. Analogously the relative nitrogen outputs were more elevated. Although both cultivars have shown significant differences in relation to positive control, the alterations were more pronounced in rats fed on Bays cultivar, which showed relative fecal dry matter and nitrogen outputs 2.8- and 5.6-fold, respectively, higher than those found in positive control. It is probable that these results are a consequence of the low protein digestibility, since the heat-treatment of Bays cultivar significantly reduced the relative fecal and nitrogen outputs by 1.8- and 3.3-fold, respectively.

The consumption of raw soybeans led to organ weight alterations (Table 6). In comparison with internal organs of egg-white-fed rats, the diets based on Rio Balsas and Bays meals induced atrophy of the thymus and spleen and enlargement of the small intestine, caecum + colon, stomach, lungs and kidneys. Additionally, Bays cultivar, but not Rio Balsas, caused a significant increase of the pancreas and liver compared with egg-white fed rats. The diet containing Bays seed meal seems to be more toxic than that formulated with Rio Balsas since alterations of the relative dry weights of internal organs of Bays seed fed rats were more pronounced. The improvement in nutritional quality following wet heat-treatment of Bays seeds was verified by elimination or attenuation of almost all organ alterations described above, suggesting the denaturation of heat-labile proteins, responsible for the observed alterations.

### *3.4. Relationships between soybean proteins and nutritional performance*

The results of the regression analyses are depicted in Table 7. These results showed that in the raw samples the

Table 6
Relative dry weights[a] (g/100 g body dry matter) of organs of rats fed on NPC, EW and experimental diets

| Organ | Diets | | | | |
|---|---|---|---|---|---|
| | NPC | EW | Rio Balsas | Bays | |
| | | | | Raw | Cooked |
| Stomach | 0.64 ± 0.03b | 0.46 ± 0.01a | 0.55 ± 0.04c | 0.61 ± 0.02b | 0.50 ± 0.03d |
| Intestine | 2.46 ± 0.02c | 1.89 ± 0.06a | 2.28 ± 0.09d | 2.71 ± 0.02b | 1.99 ± 0.05a |
| Caecum + colon | 0.53 ± 0.02d | 0.44 ± 0.01a | 0.67 ± 0.02c | 0.73 ± 0.02b | 0.51 ± 0.02d |
| Liver | 3.31 ± 0.05b | 2.93 ± 0.01a | 3.07 ± 0.27ab | 3.23 ± 0.03b | 2.94 ± 0.18a |
| Pancreas | 0.22 ± 0.02c | 0.29 ± 0.00a | 0.30 ± 0.02a | 0.36 ± 0.02b | 0.30 ± 0.03a |
| Thymus | 0.13 ± 0.01d | 0.23 ± 0.00a | 0.21 ± 0.01b | 0.18 ± 0.00c | 0.23 ± 0.01a |
| Spleen | 0.13 ± 0.01e | 0.19 ± 0.01a | 0.16 ± 0.01c | 0.14 ± 0.00d | 0.18 ± 0.00b |
| Kidneys | 0.77 ± 0.03b | 0.54 ± 0.02a | 0.62 ± 0.03c | 0.67 ± 0.02d | 0.58 ± 0.02c |
| Heart | 0.31 ± 0.02b | 0.25 ± 0.02a | 0.26 ± 0.01a | 0.27 ± 0.00a | 0.25 ± 0.02a |
| Lungs | 0.41 ± 0.01b | 0.38 ± 0.01a | 0.40 ± 0.01b | 0.41 ± 0.01b | 0.38 ± 0.01a |

[a] Values in a horizontal row with different following letters differ significantly ($P < 0.05$).

Table 7
Regression results: estimated coefficients, (T-ratio) and R-squares

| Independent variables | Dependent variables | | | |
|---|---|---|---|---|
| | Food intake | Weight gain | NPU | Digestibility |
| Trypsin inhibitor | −5.70 (−5.98)* | −2.56 (−8.25)* | −9.07 (−45.50)* | −6.50 (−21.14)* |
| Lectin | −6.89 (−2.73)* | −3.51 (−4.25)* | −12.38 (−23.38)* | −7.50 (−9.13)* |
| Toxin | −0.21 (−0.14) | 0.15 (0.31) | 0.51 (1.63) | −0.49 (−1.02) |
| Urease | −1.45 (−6.05)* | −0.66 (−8.38)* | −2.32 (−46.21)* | −1.65 (−21.33)* |
| R-square | 0.86 | 0.92 | 0.99 | 0.98 |

* Significant at $\alpha = 0.05$.

soyatoxin was not a significant explanatory variable, whereas lectin, trypsin inhibitor and, to a lesser extent, urease were significant ($p < 0.01$) explanatory variables for feed intake, weight gain, NPU and digestibility.

## 4. Discussion

Rio Balsas and Bays cultivars have similar lectin contents, 6.5 ± 1.1 and 6.4 ± 1.9 g of lectin equivalents per kg defatted meal, respectively. These values are within the range reported for some soybean cultivars which varies from 2.4 ± 0.8 to 9.6 ± 3.2 g of lectin equivalents per kg defatted meal [5]. The level of trypsin inhibitor in Rio Balsas is relatively low as observed for other soybean cultivars, such as those in Enrey and lac-4 (28.1 ± 1.1 and 30.2 ± 3.5 g trypsin inhibited/kg flour, respectively) [5]. Bays, however, has a high value comparable to those of HOL 1983 MISS and T908 SF83 cultivars (63.8 ± 1.1 and 61.4 ± 0.9 g trypsin inhibited/kg flour, respectively) [11].

It is well documented that several seed lectins are resistant to proteolysis by gut enzymes and are detrimental to rat health when orally fed, leading to impaired growth and alterations of key organs, particularly hypertrophy of small intestine [28–29]. Dietary trypsin inhibitors are blamed to be responsible for the poor digestibility of dietary protein by interference with the proper function of trypsin leading to growth inhibition and pancreatic hypertrophy [30]. In this study the regression analyses relating the antinutritional and/or toxic factors to protein quality indicators showed that the lectin and the trypsin inhibitor were the main significant explanatory variables for the protein quality indicators. However, all the nutritional parameters, except for NPU, were accounted for at the same extent by the trypsin inhibitor and the lectin. As the two soy meals did not differ from each other in amino acid composition and lectin content, the differences in the nutritional parameters could be probably due to the contents of trypsin inhibitor, soyatoxin and urease. As to the relationship of lectins and trypsin inhibitors with organ weights, the regression analysis data showed that the small intestine enlargement is mainly induced by the lectin, although trypsin inhibitor had also a significant relationship, as shown by their respective estimated coefficients (0.72 and 0.41) and T-ratios (3.37 and 20.52). Indeed, studies have reported that the small intestine enlargement is due mainly to cellular hypertrophy and hyperplasia caused by soybean agglutinin and that trypsin inhibitor has a minor contribution [5,31]. However, when the estimated coefficients and T-ratios for pancreas weight were analyzed, the lectin (−0.17 and −1.87, respectively) did not have any significant effect, contrary to what happened to the trypsin inhibitor (0.38 and 4.29, respectively). This is in agreement with previous findings [11].

Although lectins and trypsin inhibitors have been considered the most important antinutritional factors present in some seeds, it could not be ruled out that other seed proteins may contribute either directly or synergistically to the adverse effects observed upon feeding [31]. In fact, Armour et al. [5] reported that the overall contribution of the protease inhibitors or lectin to the impaired nutritional performance of animals fed soy-based diets was small and that, whilst these components alter pancreas and small intestine metabolism, other factors may be responsible for much of the growth impairment and poor utilization observed on soy feeding.

The research was then conducted to evaluate the role of the dietary soyatoxin on the performance of rats fed on diets containing soyatoxin-rich (cv. Bays) and soyatoxin-free (cv. Rio Balsas) soybean cultivars. Soyatoxin is a single protein, distinct from trypsin inhibitor and lectin, severely toxic to mice when intraperitoneally injected. It induces tonic clonic convulsions and flaccid paralysis followed by death within 24 h, depending on the doses and route used [8]. Although purified soyatoxin did not show acute toxicity when intragastrically intubated in rats, as shown previously, this would not exclude a contribution of this protein to the deleterious effects of raw soybean meals. Nevertheless, the data of regression analyses showed that in the raw samples, soyatoxin was not a significant explanatory variable for feed intake, weight gain, NPU and digestibility. On the other hand, the analysis of the relationship of soyatoxin with organ weights revealed that this protein was a significant explanatory variable for pancreas and small intestine weight, with estimated coefficients of 0.22 and 0.83, respectively, and T-ratio of 4.14 and 6.72, respectively. Thus, these results suggest that the overall contribution of soyatoxin to the impaired nutritional performance of animals fed

soybean-based diets was small, if any, and that this protein has much more importance as a toxic component of soybeans.

The effect of urease to the nutritive value of soybeans was also evaluated since the urease content varied significantly between the studied cultivars. The concern with urease in this work is justified on the basis that studies [32] have shown the association of bacterial urease with ulceration of the gastric mucosa on vertebrates, and on the observations of Polacco and Holland [33] that the embryo-specific urease (from soybean, jackbean, watermelon and many other members of Fabaceae and Curcubitaceae) might mimic the effects observed for microbial urease due to the homology (>50%) observed among the plant seed and bacterial enzymes. In this study it was verified that this enzyme is a significant explanatory variable for all the nutritional parameters and that it has relationship with the organ weights, although to a lesser extent than the other components. Its estimated coefficient and T-ratio for pancreas weight were 0.15 and 4.56, respectively, and those for small intestine weight were 0.16 and 20.78, respectively.

In conclusion, all the studied components interfere in the nutritional parameters and/or in organ size. Although the biological alterations have been mainly induced by lectin and trypsin inhibitor, we cannot exclude the contribution of other factors, such as soyatoxin and urease. These negative effects, however, can be partially eliminated or inactivated with adequate heat-treatment.

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