DOI:10.1021/jf803903h

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Combination of Chemical Analyses and Animal Feeding Trials as Reliable Procedures to Assess the Safety of Heat Processed Soybean Seeds

Ilka M. Vasconcelos,[†] Isabel Cristiane F. Brasil,[†] José Tadeu A. Oliveira,[†] Cláudio C. Campello,[‡] Fernanda Maria M. Maia,[§] Maria Verônica M. Campello,[‡] Davi F. Farias,[†] and Ana Fontenele U. Carvalho*,[∥]

[†]Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, P.O. Box 6020, Campus do Pici, 60455-970 Fortaleza, Ceará, Brazil, [‡]Faculdade de Veterinária, Universidade Estadual do Ceará, Fortaleza, Ceará, Brazil, [§]Curso de Nutrição, Universidade Estadual do Ceará, Fortaleza, Ceará, Brazil, and [∥]Departamento de Biologia, Universidade Federal do Ceará, Campus do Pici, Fortaleza, Ceará, Campus do Pici, 60455-970 Fortaleza, Ceará, Brazil

This study assessed whether chemical analyses are sufficient to guarantee the safety of heat processing of soybeans (SB) for human/animal consumption. The effects of extrusion and dry-toasting were analyzed upon seed composition and performance of broiler chicks. None of these induced appreciable changes in protein content and amino acid composition. Conversely, toasting reduced all antinutritional proteins by over 85%. Despite that, the animals fed on toasted SB demonstrated a low performance (feed efficiency 57.8 g/100 g). Extrusion gave place to higher contents of antinutrients, particularly of trypsin inhibitors (27.53 g/kg flour), but animal performance was significantly ($p < 0.05$) better (feed efficiency 63.2 g/100 g). Upon the basis of chemical analyses, dry-toasting represents the treatment of choice. However, considering the results of the feeding trials, extrusion appears to be the safest method. In conclusion, in order to evaluate the reliability of any processing method intended to improve nutritional value, the combination of chemical and animal studies is necessary.

KEYWORDS: Safety assessment; extrusion; dry-toasting; antinutritional/toxic proteins; soybeans; *Glycine max*

1. INTRODUCTION

Heat treatment of soybean seeds and byproducts is known to be required before their use as food or feed since they contain antinutritional compounds, which when ingested may cause inhibition of growth, decreased feed efficiency, pancreatic hypertrophy, and other deleterious effects to several organisms (*1*). Soybean trypsin inhibitors (SBTI) and lectins (SBA) are considered to be the major seed proteins responsible for the poor nutritional value of raw soybean meals (*2*, *3*). It has been suggested that other proteins, such as soyatoxin (SYTX), a toxin isolated from soybeans that is lethal to mice by the intraperitoneal route (*4*), and urease, might also contribute to the deleterious effects observed upon feeding soybeans (*5*).

Several methods of heat treatment have been developed to increase the nutritional quality of soybean-based products to reduce the content of antinutritional factors (ANF). Among these are wet or dry-toasting, extrusion, and infrared radiation (*6*). However, adequate inactivation processes of ANF require the accurate control of temperature, moisture, and time of application (*7*, *8*). Both under- or overheating are detrimental to the effective usage of seed nutrients (*2*, *9*). Extrusion and dry-toasting of soybeans are heat treatment procedures frequently used in developing countries because of the simple and low cost machinery necessary for these processes and also because of their capacity to inactivate ANF, mainly trypsin inhibitors and lectins (*10*). These two methods differ in terms of temperature, pressure, and time of exposure of grains to heat. Extrusion employs higher temperatures, but it is a short time process, and pressure is drastically reduced during the process, while dry-toasting is carried out at slightly lower temperatures but for a more prolonged period and constant pressure (*6*, *11*). These differences in the soybean heat treatment conditions may have a marked influence on inactivation of antinutritional compounds and nutrient digestibility, affecting protein utilization by experimental animals. Usually, the food industry shows resistance to the use of feeding studies because of the fact that they are laborious, longer, and more expensive than chemical/biochemical analyses. This study aimed to assess whether chemical and biochemical analyses per se are adequate to guarantee the safety of heat processing of soybeans (SB) for human and animal consumption or whether feeding trials are indeed necessary. Thus, extrusion and dry-toasting were analyzed regarding their effects not only upon seed nutrient/antinutrient composition but also

*Corresponding author. Tel/Fax: + 55 85 3366 9830. E-mail: aurano@ufc.br.

 Published on Web 05/15/2009

upon the performance of broiler chicks, one of the main target animals of SB-containing diets

2. MATERIALS AND METHODS

2.1. Materials. Soybeans [*Glycine max* (L.) Merr] were purchased as a mixture of several undefined cultivars from local commercial sources. Extruded soybeans were obtained by passing the seeds through a screw extruder (Ext 400 Calibrás, São Paulo, Brazil) with dry heat at 129 °C and a feed rate of 300 to 350 kg/h (corresponding to a retention time of 12−14 s). Toasted soybeans were produced by dry-heating at 100 °C and 2 kgf/cm^2 for 60 min (Tesoy, Buenos Aires, Argentina). Kunitz-type soybean trypsin inhibitor (type I−S), *N*-α-benzoyl-L-arginine *p*-nitroanilide, trypsin (type I), and urease (41H7008, 870,000 units/g) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of analytical grade.

2.2. Moisture, Protein, and Oil Determination. Protein was determined in the raw and processed soybeans by measuring the nitrogen content in Kjeldahl digests according to a manual colorimetric assay (*12*), using a nitrogen conversion factor of 6.25 Moisture and oil contents were determined according to previously established protocols (*13*).

2.3. Amino Acid Composition. Raw and processed samples (10 mg) were hydrolyzed with 6 M HCl containing 1% phenol, at 110 °C, for 20 h in sealed glass tubes under a nitrogen atmosphere. HCl and phenol were removed by evaporation, and the amino acid compositions were established after chromatography on a Biochrom 20 system (Pharmacia, Biotech, Uppsala, Sweden). Tryptophan content was measured colorimetrically by the acid ninhydrin method (*14*).

2.4. Polyacrylamide Gel Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was performed according to Laemmli (*15*). SDS−PAGE was carried out in a 3.5-mm vertical slab gel (12.0 × 17.0 cm) consisting of stacking gel mix, 3.5% total acrylamide, and main running gel mix, 17.5% acrylamide, prepared in 1.5 M Tris-HCl at pH 8.8. Samples (50 μg) were dissolved in 0.125 M Tris-HCl, pH 6.8, containing 1% SDS and 1% β-mercaptoethanol, and incubated at 100 °C for 10 min. Electrophoresis was carried out at 20 mA constant current for 2 h. Protein bands were visualized by staining with 0.05% Coomassie brilliant blue R-250.

2.5. Aqueous Soybean Crude Extracts. Samples of raw and heat-processed soybeans were ground in a coffee grinder to a fine powder, defatted with petroleum ether (10%, w/v, suspension), and air-dried at room temperature. For the preparation of the aqueous crude extract from each soybean sample, the defatted meal was suspended in 0.125 M Tris-HCl buffer, pH 7.5, in a proportion of 1:5 (w/v). The suspension was maintained under continuous stirring for 3 h at 4 °C and then filtered. The press cake was re-extracted under the same conditions. The filtrate was centrifuged at 15,000*g* for 30 min, at 4 °C and the clear supernatant dialyzed (cutoff MW 12,000) against the extracting buffer. The protein content of seed extracts was evaluated by the method described by Bradford (*16*), and bovine serum albumin was used as the standard.

2.6. Antinutritional/Toxic Proteins. The crude extracts, prepared as described above, were the starting materials for detecting hemagglutinating, toxic, and urease activities. Hemagglutinating activity was assayed according to Moreira and Perrone (*17*), using rabbit, human, and chicken erythrocytes. Toxic activity was defined as the mortality observed in mice within 24 h after intraperitoneal (ip) injections of the crude extracts (*4*). Urease assay was carried out according to the indophenol method (*18*). The protease inhibitor assay was carried out by the modified Kakade method (*19*).

2.7. Diets. Diets were formulated as shown in **Table 1** to meet the minimal nutritional requirements of broiler chicks (*20*). Experimental diets were prepared to contain the equivalent of 210 g protein/kg diet. To bring the amino acid contents to the target requirements for chicks, diets were supplemented with DL-methionine based on the amino acid contents of the soybean samples used in the present study. The diets were prepared in order to contain 12.55 MJ/kg of metabolizable energy.

2.8. Feeding Trial. As an approach to verify the effects of different processing methods on the protein quality of commercial soybeans, male broiler chicks (*Gallus gallus domesticus*) of the Ross strain, with an average weight of 53.5 g at hatching, were used as a model since they represent an important soybean-dependent economically explored animal species. The birds were fed ad libitum on a commercial ration (CR) (21% crude protein; 12.55 MJ/kg metabolically energy) until 9 days of age. They were then individually housed at room temperature, in screen-bottomed cages (0.25 × 0.50 cm), and fed the same CR for 2 more days as a period of adaptation to experimental conditions. After that, the birds were randomly assigned to four treatments (three replicates of six chicks each) corresponding to rations containing commercial soybean meal (CSBM; positive control group), raw soybean meal (RSBM; negative control group), extruded soybean meal (ESBM), and toasted soybean meal (TSBM). CSBM was purchased from a commercial source and corresponds to the wet-heated defatted soybean meal commonly used for broiler chick feed. The birds were fed on experimental diets for 12 days (from 10 to 21 days posthatching), with feed and water supplied ad libitum and light provided continuously. Diet spillage and refused diet were recorded daily. Chick body weights were recorded on alternate days to reduce the stress caused by handling. At the end of the trial, the animals were slaughtered in a slaughter house and the internal organs (proventriculus, small intestine, ceca, pancreas, and liver) dissected, blot-dried, and their fresh weights recorded. The small intestine was immediately rinsed with ice-cold phosphate buffered saline to remove any fecal particles, blot-dried, and weighed. Samples from these organs were taken or collected for histological analyses. The remaining parts of the intestine and all other internal organs were then freeze-dried, while the carcasses were dried in an oven at 70 °C for 72 h. Dry weights were recorded before incorporating the organs with their original carcasses that were then ground and kept in a desiccator for later chemical analyses. Diets and carcasses were analyzed for moisture content (*13*) and total nitrogen (*12*). The response of animals to dietary protein was determined in terms of weight gain, feed intake, feed efficiency, body nitrogen, and relative dry weight of organs. All results were calculated for each bird and the mean calculated within a group.

These studies were reviewed and approved by the appropriate Animal Care and Use Committee, assuring that all birds received humane care.

2.9. Statistical Analysis. The results were subjected to a one-way analysis of variance and the significance of difference among means

Table 1. Composition (g/kg) of Control (CSBM, RSBM) and Experimental (ESBM, TSBM) Diets[a]

	diet			
ingredient	CSBM	RSBM	ESBM	TSBM
maize starch	525.0	414.0	418.0	414.0
corn (grain)				
limestone	9.3	9.0	9.0	9.0
dicalcium phosphate	20.0	21.0	21.0	21.0
vegetable oil	30.0	—	—	—
salt	5.2	5.2	5.2	5.2
vitamin premix[b]	3.0	3.0	3.0	3.0
mineral premix[b]	0.5	0.5	0.5	0.5
soybean				
defatted, heated	440.0			
raw		540.0		
extruded			537.0	
toasted				540.0
DL-methionine	1.8	0.7	0.6	0.7
metabolizable energy (MJ/kg)	12.5	12.5	12.5	
Crude protein g/kg	215.2	215.2	215.1	
Met + Cys g/kg	9.0	9.0	9.0	
Lys g/kg	12.5	15.2	12.9	
calcium g/kg	9.6	9.7	9.7	
available phosphorus g/kg	4.4	4.5	4.5	

[a] CSBM, commercial soybean meal; RSBM, raw soybean meal; ESBM, extruded soybean meal; and TSBM, toasted soybean meal. [b] All diets were formulated to provide the following vitamins/minerals per kilogram of diet: vitamin A, 7,998 IU; vitamin D_3, 2,000 IU; vitamin E, 15 mg; vitamin K_3, 1.8 mg; vitamin B_1, 1.8 mg; vitamin B_2, 6 mg; vitamin B_6, 2.8 mg; vitamin B_{12}, 0.012 mg; pantothenic acid, 15 mg; niacin, 40 mg; folic acid, 1.0 mg; choline, 400 mg; antioxidant, 22.5 mg; selenium, 0.3 mg; manganese, 75 mg; iron, 50 mg; copper, 8 mg; zinc, 50 mg; iodine, 0.75 mg.

Table 2. Protein and Oil Determination (g/kg Seed) of Raw and Processed Soybeans

	samples[a]			
components	CSBM	RSB	ESB	TSB
moisture	126.5 ± 1.3 a	107.8 ± 2.2 b	74.1 ± 0.9 c	105.9 ± 3.2 b
protein[b]	558.9 ± 9.2 a	414.9 ± 15.3 b	397.5 ± 7.8 b	399.4 ± 10.4 b
oil	47.9 ± 5.6 b	261.9 ± 24.0 a	257.0 ± 16.0 a	264.7 ± 14.5 a

[a] Data represent mean and standard deviation of six independent determinations based on seed flour dry matter. Values with different letters in the same row differ statistically ($p < 0.05$). CSBM, commercial soybean meal (defatted, heated); RSB, raw soybean; ESB, extruded soybean; and TSB, toasted soybean. [b] $N \times 6.25$.

determined by Tukey's honest test. The statistically significant difference was defined as $P < 0.05$.

3. RESULTS

3.1. Moisture, Protein, and Oil Determination. The moisture, protein, and oil contents were similar for the soybean samples in the raw (RSB), dry toasted (TSB), or extruded (ESB) state (**Table 2**).

3.2. Amino Acid Composition. Before preparing the diets containing the soybean seed meals as protein sources, the amino acid composition of each material was determined (**Table 3**) in order to eliminate the effects, if present, of the deficiency of these constituents. Small numerical differences in amino acid levels were noted among the different soybean materials evaluated in the present research. However, in general, the conditions of processing employed were insufficient to cause significant alterations in the amino acid composition. RSB, ESB, and TSB showed high levels of glutamine/glutamic acid and asparagine/aspartic acid constituting together about 37% of the total amino acid content.

3.3. Polyacrylamide Gel Electrophoresis. The electrophoretic patterns of the seed proteins from raw and processed soybean flours treated with SDS and β-mercaptoethanol resemble each other qualitatively since similar motilities among the corresponding protein bands were observed regardless of the soybean sample considered. The relative molecular masses varied from about 20 to 90 kDa, and the bands corresponding to the lectin (30 kDa) and trypsin inhibitor (21 kDa) were well visualized. However, quantitative differences in protein bands among the soybean samples were detected. RSB and ESB exhibited protein bands that were more prominent than those of TSB (**Figure 1**).

3.4. Antinutritional/Toxic Proteins. The soybean trypsin inhibitory, toxic, urease, and lectin activities are depicted in **Table 4**. The crude extracts from all soybean samples were able to inhibit trypsin. However, there were huge differences among the soybean materials evaluated with values varying from 4.75 to 38.80 g trypsin inhibited/kg flour. RSB followed by ESB presented the highest values of approximately 8- and 5-fold, respectively, those found for TSB. The crude aqueous extract of one (RSB) out of the four soybean samples tested was highly toxic to mice when injected by the ip route. The typical effects observed included dyspnoea and tonic−clonic convulsions preceding the death of the animals. In contrast, none of the crude extracts from processed soybeans were lethal when injected ip, even using a dose (1.83 g/kg mouse body weight) 10 times higher than the one used from RSB. Urease activity was found to be present in all soybean samples and was found at a level of 1.04 g urease per kg flour in RSB and varied from 0.16 to 0.46 g urease per kg flour for the heat-processed soybeans, suggesting that urease was significantly reduced upon heat treatment. The hemagglutinating activity measured against rabbit erythrocytes (**Table 4**) was significantly different among the studied soybean samples.

Table 3. Amino Acid Composition (g per 16 g of N) of Raw and Processed Soybeans

	samples[a]			
amino acid	CSBM	RSB	ESB	TSB
Asx	14.23	12.00	13.94	14.48
Thr[b]	3.21	3.66	3.35	3.37
Ser[b]	3.53	4.06	3.70	3.84
Glx	22.82	21.17	23.46	21.69
Pro	5.12	5.72	4.85	5.08
Gly[b]	3.31	3.70	3.39	3.48
Ala	4.32	4.56	4.54	4.46
Cys[b,c]	0.72	0.72	0.69	0.72
Met[b]	1.52	1.41	1.45	1.49
Val[b]	4.20	4.62	4.30	4.32
Ile[b]	4.33	4.63	4.16	4.20
Leu[b]	7.18	7.50	6.96	6.82
Tyr[b]	3.90	4.07	3.96	4.02
Phe[b]	5.07	5.33	5.03	5.21
His[b]	2.48	2.55	2.36	2.61
Lys[b]	5.52	5.76	5.53	5.62
Arg[b]	7.99	7.88	7.76	8.06
Trp[b]	0.55	0.66	0.57	0.53

[a] Each value represents the mean of three replicates. Standard deviations are omitted for clarity. The values are not significantly different among the soybean samples ($p > 0.05$). CSBM, commercial soybean meal; RSBM, raw soybean meal; ESBM, extruded soybean meal; and TSBM, toasted soybean meal. [b] Essential amino acid for broiler chicks. [c] Cystine.

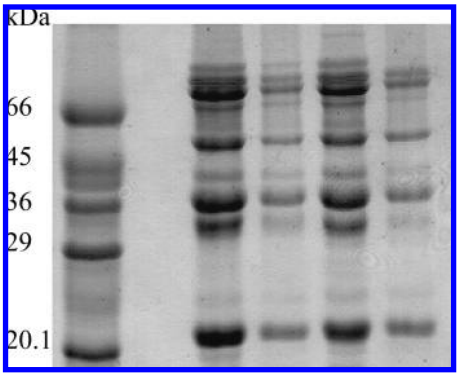

Figure 1. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis. Lane 1, molecular mass markers (bovine serum albumin, egg albumin, carbonic anhydrase, glyceraldehyde-3-phosphate dehydrogenase, and soybean trypsin with apparent molecular masses of 66, 45, 36, 29, and 20.1 kDa, respectively); lane 2, raw soybean; lane 3, toasted soybean; lane 4, extruded soybean; and lane 5, commercial soybean meal.

Further treatment of the red cells with trypsin increased the agglutinating activity of all extracts at least 8 times. As expected, the lectin content in RSB was found to be much higher than that of processed soybeans. Toasting was the most effective heat treatment for the reduction of lectin content. Interestingly, hemagglutinating activity was also detected using human erythrocytes, but only in RSB and after the red cells had been treated with trypsin. Lectin activity using A^+, B^+, or O^+ erythrocytes corresponded to 5.52, 5.52, and 11.05 hemagglutination units (HU) per kg flour, respectively. Conversely, in chicken erythrocytes, the crude extracts from raw and processed soybeans were not able to promote agglutination of these cells even when they were treated with trypsin (data not shown in **Table 4**).

3.5. Nutritional Parameters. The diets containing processed soybean meals (CSBM, ESBM, and TSBM) were much better accepted by the chicks than the RSBM diet (**Table 5**). Feed intake for CSBM was significantly higher than that of birds fed on

Table 4. Trypsin Inhibitory, Toxic, Urease and Lectin Activities Present in the Crude Extracts of the Raw and Processed Soybeans

	samples[a]			
activities	CSBM	RSB	ESB	TSB
trypsin inhibitory[b]	4.91 ± 0.37 c	38.80 ± 0.35 a	27.53 ± 1.29 b	4.75 ± 0.26 c
acute toxic[c]	NL[d]	0.183 ± 0.09 a	NL	NL
urease[e]	0.18 ± 0.01 c	1.04 ± 0.03 a	0.46 ± 0.02 b	0.16 ± 0.01 c
lectin[f] ($\times 10^4$)				
untreated	22.94 ± 1.05 c	354.58 ± 2.82 a	43.87 ± 0.92 b	10.59 ± 0.93 d
trypsin treated	184.47 ± 6.32 c	2 835.87 ± 9.88 a	352.44 ± 5.22 b	173.02 ± 8.24 d

[a] Data represent the mean and standard deviation of three replicates. Values with different letters in the same row differ statistically ($p < 0.05$). CSBM, commercial soybean meal; RSBM, raw soybean meal; ESBM, extruded soybean meal; and TSBM, toasted soybean meal. [b] Trypsin inhibitory activity is expressed as g of trypsin inhibited per kg of flour. [c] 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. [d] Not lethal even at a dose of 1.83 g per kg of mouse body weight. [e] Urease activity is shown as g of urease per kg of flour. [f] Lectin activity is expressed as hemagglutination unit (HU) per kg of flour. One HU represents the reciprocal of the highest dilution giving visible agglutination of the rabbit erythrocytes. Specific hemagglutinating activity was taken in relation to the seed crude protein content (**Table 2**).

Table 5. Nutritional Parameters of Chicks Fed on Raw and Processed Soybeans

	diets[a]			
nutritional parameters	CSBM	RSBM	ESBM	TSBM
weight gain[b] (g)	435.3 ± 25.4 a	249.0 ± 17.5 c	450.5 ± 14.7 a	358.8 ± 23.0 b
feed intake[b] (g)	731.8 ± 25.9 a,b	622.7 ± 24.8 d	702.5 ± 22.5 b,c	677.5 ± 18.3 c
feed efficiency[c] (g/100 g)	60.7 ± 3.7 a,b	45.1 ± 4.7 c	63.2 ± 2.9 a	57.8 ± 2.4 b
body nitrogen[b] (g/kg)	84.6 ± 3.2 a	81.1 ± 1.1 b	77.6 ± 1.6 b	78.2 ± 3.9 b

[a] Data represent the mean and standard deviation of three replicates of six chicks from 10 to 21 days. Values with different letters in the same row differ statistically ($p < 0.05$). CSBM, commercial soybean meal; RSBM, raw soybean meal; ESBM, extruded soybean meal; and TSBM, toasted soybean meal [b] Per chick. [c] Calculated in g of weight gain per 100 g of feed intake.

Table 6. Relative Dry Weights (g/100 g Body Dry Matter) of Organs of Chicks Fed on Raw and Processed Soybeans

	diets[a]			
organ	CSBM	RSBM	ESBM	TSBM
proventriculus	6.10 ± 0.49 b,c	5.75 ± 0.54 b,c	7.65 ± 0.51 a	5.18 ± 0.53 c
liver	2.28 ± 0.09 a	1.90 ± 0.14 b	2.13 ± 0.12 a,b	1.89 ± 0.12 b
pancreas	2.75 ± 0.11 c	7.22 ± 0.64 a	3.74 ± 0.15 b	2.79 ± 0.16 c
small intestine	2.55 ± 0.22 b	3.01 ± 0.12 a	2.40 ± 0.09 b	2.31 ± 0.18 b
ceca	0.61 ± 0.02 b	0.83 ± 0.03 a	0.65 ± 0.03 b	0.67 ± 0.01 b

[a] Values with different letters in the same row differ statistically ($p < 0.05$). CSBM, commercial soybean meal; RSBM, raw soybean meal; ESBM, extruded soybean meal; and TSBM, toasted soybean meal.

RSBM and TSBM. Overall, the heat-processed soybean-fed chicks had much higher values of weight gain compared with data for chicks fed on RSBM. However, animals fed on the TSBM diet gained less weight than those fed ESBM and CSBM. Heat-processing of soybeans increased the feed efficiency relative to raw soybeans, and the feed efficiency for ESBM was significantly higher than that for TSBM.

The consumption of raw soybeans led to organ weight alterations (**Table 6**). The diet based on RSBM induced an enlargement of the pancreas, small intestine, and ceca compared to the positive control group (CSBM). The improvement in the nutritional quality following heat-processing of the soybean samples was verified by elimination or attenuation of almost all organ alterations described in the present study. However, the ESBM diet promoted an increase in pancreas and proventriculus weights when compared with the internal organs of the TSBM-fed chicks. In this respect, the diet containing ESBM seems to be less beneficial than those formulated with TSBM and CSBM since alterations in the relative dry weights of internal organs of ESBM-fed chicks were more pronounced. With regard to the histological analyses, the results did not show structural alterations or tissue damage in the internal organs, with the exception of the pancreas of RSBM-fed chicks, which presented hypertrophy of acinar cells (data not shown).

4. DISCUSSION

Because of the presence of certain ANF/toxic components in soybeans, particularly proteins, attempts to increase the utilization of this legume have employed a wide range of processing techniques. Full-fatty commercial soybeans in the raw state (RSB) or those submitted to dry-extrusion (ESB) or dry-toasting (TSB) were investigated here with regard to their protein and oil contents, amino acid composition, antinutritional/toxic protein concentrations, and the performance of broiler chicks fed on these diets (**Table 1**) as the only source of dietary protein. This study intended to produce relevant data to establish the necessity of running feeding trials to demonstrate the effectiveness of heat treatments used to increase the nutritional attributes of soybeans. To reach this goal, broiler chicks were used as an experimental model since, in Brazil as well as in many other countries, soybeans are by far the main protein source in the poultry diet.

The data for protein and oil contents (**Table 2**) are similar to those previously reported for soybeans by Vasconcelos et al. (*21*). Overall, there were no significant differences in these constituents among raw and processed soybeans, showing that the different types of heat processing did not interfere with the chemical composition of the whole seed.

The results of amino acid composition (**Table 3**) for the raw and processed soybean meals did not present great differences, showing that the heat-processing conditions employed in the current study were insufficient to cause significant alterations in the amino acid profiles. Taking lysine as a standard amino acid, its levels after the heat treatments were similar to those of unheated soybean samples. These findings are comparable to previously reported data (*22*).

Despite the similarities in the protein contents and amino acid composition observed in raw and processed soybeans, differences in the intensities of the protein bands were detected when the same amounts of samples were applied to SDS−PAGE (**Figure 1**). Both RSB and ESB exhibited more prominent protein bands than those of TSB, suggesting that protein denaturation/degradation might have occurred when commercial soybeans were submitted to dry-toasting treatments.

The nutritional value of soybean meal is usually much lower than that expected, upon the basis of its protein content and amino acid profile. This result has been mainly attributed to the presence of SBTI and SBA, which cause a series of harmful local and systemic reactions, placing these classes of molecules as antinutritive proteins (*2*, *23*).

The trypsin inhibitor content of RSB was within the range of published results (*21*). Reductions, to various extents, in the trypsin inhibitor content depended upon the heating conditions to which the raw commercial soybeans were exposed (**Table 4**). There was a reduction of 87% in the trypsin inhibitor content of TSB. In contrast, the extrusion process caused a reduction of only 29%. These figures agree with the protein patterns obtained after SDS−PAGE (**Figure 1**, 20.1 kDa band). Such an incomplete inactivation of trypsin inhibitors might occur due to the fact that despite the high temperatures employed by extrusion, it is a very fast process. Leeson and Atteh (*24*) verified that even by submitting soybean to extruding temperatures varying from 80 to 140 °C, the contents of trypsin inhibitors were not reduced to an acceptable level (<10 mg/g) that would allow a larger inclusion of soybean in broiler chick diets.

Marked lectin inactivation was obtained by heating raw soybean (**Table 4**). The dry-toasting process was the most effective in reducing the hemagglutinating activity, as compared with the extrusion method. Reductions of over 90% in lectin levels were observed in TSB when trypsin-treated or untreated rabbit erythrocytes were used. In contrast to observations with trypsin inhibitors, about a 90% reduction in the hemagglutinating activity was caused by the extrusion method. These findings demonstrate that the hemagglutinating activity was clearly more sensitive to heat treatment than the trypsin inhibitory activity.

Vasconcelos et al. (*5*) have shown that other proteins present in soybeans, such as soyatoxin and urease, might be involved with the deleterious effects observed by feeding growing rats on raw soybean based diets. These diets promoted poor nutritional parameters together with alterations in the weights of the pancreas and small intestine. Soyatoxin is a protein that is severely toxic to mice and rats when injected intraperitoneally (*4*). In contrast to trypsin inhibitors, this toxin is a highly heat-labile protein. Thus, in the present study only RSB maintained its ability to kill mice after ip injection, with the typical nervous symptoms previously described (*4*).

Urease has been used for a long time as a predictor of trypsin inhibitor content in soybeans (*7*) since Caskey and Knapp (*25*) described a positive correlation between urease and trypsin inhibitor activities. Besides, the effect of dietary urease on the nutritive value of soybeans has been evaluated by our group. The reasoning for such an investigation is based on the observations that the embryo-specific urease from plants might mimic the effects of microbial urease because of the similarity in their primary structures (*26*). Furthermore, there is an association of bacterial urease with ulceration of the gastric mucosa in vertebrates (*27*). In the present study, this protein was also sensitive to the heating processes, although to a lesser extent, when compared to soyatoxin. The lowest inactivation was obtained by extrusion. Reductions in the urease activity of ESB and TSB were 56% and 85%, respectively. The dry matter content of soybeans has a significant effect on the temperature required to appropriately denature the trypsin inhibitors and to reduce urease activity. This occurs because heat transfer is more efficient in the soybean with high moisture content than in the soybean with low moisture due to the excellent conductivity of heat in water (*6*).

As already explained, the dry-extrusion method exhibited the lowest potential for inactivating the trypsin inhibitors, urease and lectin of soybeans. Accordingly, there was an expectation for a poor performance of broiler chicks fed on the ESBM diet. Nevertheless, the chicks fed on the ESBM diet presented feed intake, weight gain, and feed efficiency values similar to those of chicks fed on the positive control CSBM diet, while the animals treated with the TSBM diet did not follow the same trend (**Table 5**). According to Clarke and Wiseman (*3*), if SBTI were reduced upon heat treatment, a factor other than trypsin inhibitor contents of full-fatty soybean samples might have played an important role in these results. As a matter of fact, it has already been reported that factors other than those usually claimed as responsible for the low nutritional value of legume seeds for growing chickens, i.e., phytates, tannins, lectins, and protease inhibitors, must be taken into account (*28*, *29*). The effects of undigestible materials should also be investigated in this context. A possible reduction of nutrient availability caused by dry-toasting at the conditions it was carried out can be speculated. In fact, heat intensity can influence protein solubility, which is an important parameter used to characterize the protein quality of soybeans (*6*, *8*).

Consumption of the RSBM diet led to hypertrophy of the pancreas (**Table 6**). A significant increase in the pancreas dry weight of chicks fed on the ESBM diet, in comparison with those of TSBM- and CSBM-fed chicks, was observed. It has been reported that pancreatic hypertrophy is caused by an increase in enzyme secretion, especially of trypsin and chymotrypsin, by the pancreas in an attempt to overcome the effects of trypsin inhibitors. Pancreatic enlargement in chicks and other animals caused by feeding on raw soybeans has been described in several works (*3*, *30*). Thus, the enlargement of the pancreas in ESBM-fed chicks is apparently, at least in part, associated with a high content of trypsin inhibitors still present in extruded soybeans.

Upon the basis of the chemical and biochemical analyses, the heat treatment of choice would be dry-toasting. However, after considering the results of feeding trials, extrusion appears to be the safest method. In conclusion, results showed that to reliably assess any processing method intended to improve the nutritional attributes of soybeans, the combination of chemical and biochemical analyses with animal feeding trials is necessary.

LITERATURE CITED

(1) Palacios, M. F.; Easter, R. A.; Soltwedel, K. T.; Parsons, C. M.; Douglas, M. W.; Hymowitz, T.; Pettigrew, J. E. Effect of soybean variety and processing on growth performance of young chicks and pigs. *J. Anim. Sci*. **2004**, *82*, 1108–1114.

(2) Liener, I. E. Implications of antinutritional components in soybean foods. *Crit. Rev. Food Sci. Nutr*. **1994**, *34*, 31–67.

(3) Clarke, E.; Wiseman, J. Effects of variability in trypsin inhibitor content of soya bean meals on true and apparent ileal digestibility of amino acids and pancreas size in broiler chicks. *Anim. Feed Sci. Technol*. **2005**, *121*, 125–138.

(4) Vasconcelos, I. M.; Trentim, A.; Guimarães, J. A.; Carlini, C. R. Purification and physicochemical characterization of soyatoxin, a novel toxic protein isolated from soybeans (*Glycine max*). *Arch. Biochem. Biophys*. **1994**, *312*, 357–366.

(5) Vasconcelos, I. M.; Maia, A. A. B.; Siebra, E. A.; Oliveira, J. T. A.; Carvalho, A. F. F. U.; Melo, V. M. M.; Carlini, C. R.; Castelar, L. I. M. Nutritional study of two Brazilian soybean (*Glycine max*)

cultivars differing in the contents of antinutritional and toxic proteins. *J. Nutr. Biochem*. **2001**, *12*, 55–62.
(6) Wiriyaumpaiwong, S.; Soponronnarit, S.; Prachayawarakorn, S. Comparative study of heating processes for full-fat soybeans. *J. Food Eng*. **2004**, *65*, 371–382.
(7) Olguin, M. C.; Hisano, N.; D'Ottavio, A. E.; Zingale, M. I.; Revelant, G. C.; Calderari, S. A. Nutritional and antinutritional aspects of an Argentinian soy flour assessed on weanling rats. *J. Food Comp. Anal*. **2003**, *16*, 441–449.
(8) Rehman, Z.; Shah, W. H. Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chem*. **2005**, *91*, 327–331.
(9) Suresh, D.; Manjunatha, H.; Srinivasan, K. Effect of heat processing of spices on the concentrations of their bioactive principles: Turmeric (*Curcuma longa*), red pepper (*Capsicum annum*) and black pepper (*Piper nigrum*). *J. Food Comp. Anal*. **2007**, *20*, 346–351.
(10) Mendes, W. S.; Silva, I. J.; Fontes, D. O. Composição química e valor nutritivo de soja crua e submetida a diferentes processamentos técnicos para suínos em crescimento. *Arq. Bras. Med. Vet. Zootec*. **2004**, *56*, 207–213.
(11) Orias, F.; Aldrich, C. G.; Elizalde, J. C.; Bauer, L. L.; Merchen, N. R. The effects of dry extrusion temperature of whole soybeans on digestion of protein and amino acids by steers. *J. Anim. Sci*. **2002**, *80*, 2493–2501.
(12) Baethgen, W. E.; Alley, M. M. A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant Kjeldahl digests. *Commun. Soil Sci. Plant Anal*. **1989**, *20*, 961–969.
(13) AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed.; Helrich, K., Ed.; AOAC: Arlington, VA, 1990; pp 79, 777.
(14) Pintér-Szakács, M.; Molnár-Perl, H. Determination of tryptophan in unhydrolyzed food and feedstuffs by the acid ninhydrin method. *J. Agric. Food Chem*. **1990**, *38*, 720–726.
(15) Laemmli, U. K. Cleavage of structural proteins during the assembly of bacteriophage T4. *Nature* **1970**, *227*, 685–689.
(16) Bradford, M. M. A rapid and sensitive method for the quantification of microgram quantities for proteins utilizing the principle of protein-dye binding. *Anal. Biochem*. **1976**, *72*, 248–254.
(17) Moreira, R. A.; Perrone, J. C. Purification and partial characterization of a lectin from *Phaseolus vulgaris*. *Plant Physiol*. **1977**, *59*, 783–787.
(18) Kaplan, A. The Determination of Urea, Ammonia and Urease. In *Methods of Biochemical Analysis*; John Wiley & Sons: New York, 1969; pp 311−324.
(19) Hamerstrand, G. E.; Black, L. T.; Glover, J. D. Trypsin inhibitors in soy products: modification of the standard analytical procedure. *Cereal Chem*. **1981**, *58*, 42–45.
(20) Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L. *Tabelas Brasileiras Para Aves e Suínos: Composição de Alimentos e Exigências Nutricionais*. Horácio Rostagno: Viçosa, Brazil, 2000; p 141.
(21) Vasconcelos, I. M.; Siebra, E. A.; Maia, A. A. B.; Moreira, R. A.; Campelo, G. J. A.; Oliveira, J. T. A. Composition, toxic and antinutritional factors of newly developed cultivars of Brazilian soybean (*Glycine max*). *J. Sci. Food Agric*. **1997**, *75*, 419–426.
(22) Karr-Lilienthal, L. K.; Grieshop, C. M.; Merchen, N. R.; Mahan, D. C.; Fahey, G. C.Jr. Chemical composition and protein quality comparisons of soybeans and soybean meals from five leading soybean-producing countries. *J. Agric. Food Chem*. **2004**, *52*, 6193–6199.
(23) Bajpai, S.; Sharma, A.; Gupta, M. N. Removal and recovery of antinutritional factors from soybean flour. *Food Chem*. **2005**, *89*, 497–501.
(24) Leeson, S.; Atteh, J. O. Response of broiler chicks to dietary full-fat soybeans extruded at different temperatures prior to or after grinding. *J. Sci. Technol*. **1996**, *57*, 239–245.
(25) Caskey, C. D.; Knapp, F. Method for determining inadequately heated soybean meal. *Ind. Eng. Chem*. **1944**, *16*, 640–641.
(26) Polacco, J. C.; Holland, M. A. Roles of urease in plant cells. *Int. Rev. Cytol*. **1993**, *145*, 65–103.
(27) Cussac, V.; Ferrero, R. L.; Labigne, A. Expression of *Helicobacter pilori* urease genes in *Escherichia coli* grown under nitrogen-limiting conditions. *J. Bacteriol*. **1992**, *174*, 2466–2473.
(28) Rubio, L. A.; Brenes, A.; Castaño, M. Histological alterations of the pancreas and the intestinal tract produced by raw faba beans (*Vicia faba* L. minor) diets in growing chicks. *Br. Poultry Sci*. **1989**, *30*, 15–28.
(29) Rubio, L. A.; Brenes, A.; Castaño, M. The utilization of raw and autoclaved faba beans (*Vicia faba* L., var. minor) and faba bean fractions in diets for growing broiler chickens. *Br. J. Nutr*. **1990**, *63*, 419–430.
(30) Grant, G.; Dorward, P. M.; Buchan, W. C.; Armour, J. C.; Pusztai, A. Consumption of diets containing raw soya beans (*Glycine max*), kidney beans (*Phaseolus vulgaris*), cowpeas (*Vigna unguiculata*) or lupin seeds (*Lupinus angustifolius*) by rats for up to 700 days. Effects on body composition and organ weights. *Br. J. Nutr*. **1995**, *73*, 17–29.

Received December 16, 2008. Revised Manuscript Received March 24, 2009. This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Programa de Apoio ao Desenvolvimento Científico Tecnológico (PADCT) and Fundação de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP).