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Nutritional Responses of Rats to Diets Based on Chickpea (*Cicer arietinum* L.) Seed Meal or Its Protein Fractions

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The aim of this study was to isolate the protein fractions from chickpea, var. IAC-Marrocos, as well as to evaluate its in vivo nutritional protein quality. Among the proteins, albumins showed better nutritional value in the in vivo assays and amino acid contents, despite their higher trypsin inhibitor contents. Trypsin inhibitors were found to be heat labile in all samples, but the digestibility results for unheated and heated flour and albumins suggest that their contents are not very decisive. The PER values for casein (not supplemented) were very similar to those of heated flour and unheated or heated albumin and total globulins. The albumin and glutelin fractions showed the best results for PDCAAS, however, lower than those of casein. Despite the high digestibility of the globulin the very low essential amino acid content lowered its PDCAAS, and it had the lowest values.

KEYWORDS: Chickpea; protein fractions; biological assays; protein quality; protein digestibility; PDCAAS

INTRODUCTION

Plant proteins are increasingly being used as an alternative to proteins from animal sources in human nutrition. Among plants, legume seeds such as soybean, common beans, chickpeas, lupins, or lentils represent a rich source of proteins, carbohydrates, several water-soluble vitamins, and minerals (*1*). Plant proteins have been reported as less susceptible to hydrolysis than animal proteins. This fact and the low sulfur amino acid content have been pointed to as being responsible for the low nutritional value of legume proteins (*1, 2*). The presence of antinutritional compounds, such as protease and amylase inhibitors and lectins, in legume grains and the structural properties of native storage proteins are two factors that may cause the limited digestibility of legume proteins (*1*−*4*). Heat treatment has been described as reducing the activity of protease inhibitors and leading to denaturation of the protein, with consequent increase in its digestibility (*2, 3*), but heat could also cause the opposite effect, leading to protein aggregation or rearrangement that would affect the enzymatic hydrolysis (*3*).

Legume species that have high protein contents and adapt easily to varied soil and climate conditions have been the object of studies in several countries (*5*). Among the food legume grains, chickpea seeds (*Cicer arietinum* L.) are considered to be an important protein source, presenting high nutritional quality in comparison with other food legumes (*4, 5*); however, both the quantity and quality of protein vary considerably depending on soil and climatic conditions (*6*). It is therefore interesting to investigate cultivars grown in different regions (*7*). IAC-Marrocos is a variety developed by the Institute of Agronomy of Campinas, Brazil, for adaptation to the soil and climate conditions and to supply the increasing demand for the grain.

In this sense, the objectives of this study were to evaluate nutritional features of protein from the IAC-Marrocos variety through in vivo rat assays. Some factors, such as protease inhibitor contents, amino acid composition, and effects of heat treatment were observed. All determinations were carried out on total protein and its fractions separately.

MATERIALS AND METHODS

Materials. Chickpea seeds (*Cicer arietinum* L.), cv. IAC-Marrocos, were supplied by Instituto Agronômico de Campinas, São Paulo, Brazil. Casein was purchased from Sigma Chemical Co., St. Louis, MO. Soy oil and maize starch were purchased from the local market. Other chemicals were of reagent grade. The seeds were soaked in distilled water (4 °C/12 h), decorticated, air-dried, and powdered to 60-mesh sieve. The flour was defatted by shaking with hexane (1:6 w/v) for about 4 h at room temperature, and after a change of the solvent, the process was repeated during an additional 2 h, followed by drying to room temperature, and then used for further extractions and analysis.

Methods. *Isolation of Protein Fractions.* Albumin, total globulin, prolamin, and glutelin protein fractions were extracted from defatted flour as described by Neves and Lourenco (*4*). The flour sample was extracted three times with 0.5 mol/L NaCl solution (1:10 w/v) by shaking for 1 h at

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4 °C and centrifuged at 9000*g* for 60 min. The combined supernatants, containing salt-soluble proteins, were saved, and the residue was extracted with 70% ethanol and reextracted twice with 0.1 mol/L NaOH solution to separate the prolamin and glutelin fraction, respectively, by shaking for 1 h at 4 °C and centrifugation at 9000*g* for 60 min. Salt-soluble proteins, in the supernatant, were separated into albumin and globulin fractions by dialysis against distilled water. Albumins remained in the supernatant, and the globulins were obtained as a precipitate after centrifugation (5000*g* for 30 min). The major globulin was separated from total globulin precipitate as described by Kumar and Venkataraman (*8*). The total globulin sample was dissolved in 100 g/L NaCl (1:20 w/v), and this solution was diluted 10 times with distilled water. After its centrifugation (5000*g*/30 min), the supernatant containing vicilin-type globulin was saved, and the precipitate was again dissolved in 100 g/L NaCl (1:20 w/v) and diluted 20 times with distilled water. The major globulin (legumin-type) was then collected by centrifugation (5000*g*/30 min). Globulin fraction precipitates containing salt (NaCl) were resuspended and dialyzed against distilled water. Glutelin extract, containing residual NaOH, was neutralized and dialyzed against distilled water. All extracts were frozen (−18 °C) and freeze-dried.

Heat Treatment. A portion of defatted flour and protein fractions were suspended in distilled water (1:6 w/v), autoclaved at 121 °C for 15 min, and freeze-dried.

Nitrogen Determination. Nitrogen was determined according to the Kjeldahl method (*9*). Crude protein was calculated as N × 6.25 for chickpea proteins and N × 6.38 for the casein sample.

Chemical Composition. Moisture, fat, and ash contents of chickpea seeds and flours were determined according to AOAC methods (*9*). Crude fiber content was estimated as acid detergent fiber fraction. Carbohydrate level was estimated by difference.

Trypsin Inhibitors. Trypsin inhibitors were measured as described by Kakade et al. (*10*), using benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) as substrate. One trypsin unit (TU) is arbitrarily defined as an increase of 0.01 absorbance unit at 410 nm. Results are expressed as the number of trypsin units inhibited (TIU) per milligram of protein fraction and per milligram of sample (flour as-is basis).

Carbohydrates. The carbohydrate content was determined by suspending the samples in 100 g/L trichloroacetic acid (TCA) and centrifuging (7000*g*/15 min) to eliminate nonprotein carbohydrates. The precipitate was used to determine carbohydrates, as described by Dubois et al. (*11*), with glucose as a standard. All assays were performed in triplicate.

Amino Acid Analysis. Amino acids in defatted flour and protein fractions were determined with a Dionex-DX300 analyzer after protein hydrolysis at 110 °C for 22 h in 6 N HCl, under vacuum. Tryptophan residues were determined after Pronase hydrolysis of samples and reaction with *p*-dimethylaminobenzaldehyde.

Biological Trials. Male weanling Wistar rats, weighing 50 ± 3.0 g, were fed standard laboratory rat chow for an acclimatization period of 2 days. After this period, animals were randomly divided into 11 experimental groups of 8 rats each. They were housed in individual metabolic cages, in a room maintained at 24 ± 1 °C and 50−60% relative humidity, with a 12 h light/dark cycle. Modified diets were formulated according to the AIN-93 diet for rat growth (*12*), except for the protein content. The diets were composed of sample protein, 8 g/100 g; sucrose, 10 g/100 g; fat, 7 g/100 g; vitamin mix, 1 g/100 g; mineral mix, 3.5 g/100 g; fiber, 5 g/100 g; choline bitartrate, 0.25 g/100 g; and cornstarch to make up 100 g. Casein control diet was supplemented with 0.3 g/100 g L-cystine. One experimental group was fed a protein-free diet. Feed and water were provided ad libitum. The rats were fed for 14 days to determine the protein efficiency ratio (PER). The apparent and true digestibility and biological value of each protein were also determined with the same experimental groups, as described by McDonough et al. (*13*). Feces and urine were collected daily between the fifth and ninth days of the experiment. The feces were dried in a hot-air oven at 100 °C, cooled, weighed, and ground in a mortar. To minimize urine contamination with protein from diet residues, the urine samples were precipitated with 100 g/L TCA and centrifuged (7000*g*/15 min), and the precipitate was adjusted to a known volume. The nitrogen content in the feces and urine was determined according to the Kjeldahl method (*9*).

Table 1. Chemical Composition of the Chickpea Flours (*Cicer arietinum* L. Var. IAC Marrocos)

component	whole flour (g/100 g of sample)[a]	decorticated flour (g/100 g of sample)[a]	decorticated and defatted flour (g/100 g of sample)[a]
moisture	13.16 ± 0.33	9.26 ± 0.12	10.60 ± 0.22
protein	17.62 ± 0.90	22.86 ± 0.52	25.01 ± 0.42
fat	5.09 ± 0.02	7.32 ± 0.01	0.38 ± 0.02
ash	2.71 ± 0.03	2.49 ± 0.04	2.40 ± 0.02
fiber	6.02 ± 0.59	1.23 ± 0.17	2.11 ± 0.03
total carbohydrate[b]	55.40	57.02	59.50

[a] Values are means ± SD, $n = 3$. [b] Obtained by difference.

Protein Digestibility Corrected Amino Acid Scoring (PDCAAS). The PDCAAS was calculated on the basis of the essential amino acid (EAA) requirements for adults, by the method used in the Sarwar and McDonough (*14*) study, using the in vivo true protein digestibility results to corrections.

$$\text{PDCAAS} = (\text{amino acid content in food protein} \times \text{true digestibility}) \div \text{amino acid content of reference pattern}$$

Statistical Analyses. All assays were performed in triplicate, except the rat assay, $n = 8$, and expressed as means ± SD. Analysis of variance (ANOVA) was used to compare group results ($p \leq 0.05$).

RESULTS AND DISCUSSION

The chemical compositions of chickpea flours are shown in **Table 1**. Chickpea seeds (whole flour) were found to contain 17.62 g/100 g of flour. This result agrees with those of Singh et al. (*6*), who found the protein content ranged from 16.1 to 30.3 g/100 g in chickpea grown at different locations. In addition to genetic makeup, certain environmental factors such as location, soil type, irrigation, and fertilization affect the protein content in chickpea (*5*).

The coat of chickpea seeds and fat in the flour were extracted to reduce the interference of fiber and fat in the protein isolation and assays. The seed coat represented about 6.4 g/100 g of the seeds' weight, and the decortication procedure (removal of the coat) was reflected in an increase in protein and fat percentages and a decrease in ash content in chickpea flour (**Table 1**). Similar results were reported by Attia et al. (*15*), who found seed coat percentages of 6.7, 6.8, and 9.6 in three chickpea cultivars. The same authors presented very similar results for protein (20.7 and 21.4%), ether extract (6.41 and 6.77%), ash (3.96 and 3.54%), moisture (11.41 and 10.61%), and acid detergent fiber component (5.32 and 1.80%) of whole and decorticated chickpea flour, respectively.

The decorticated and defatted chickpea flour showed protein content of 25.01 g/100 g and, as in other legumes, the globulin fraction was the major constituent of chickpea protein (**Table 2**). The total globulins were fractionated in two principal fractions (*16*), and the legumin-like (11S-type) was the major fraction, as shown in **Table 2**. The isolated protein fractions albumin, total and major globulin, and glutelin obtained in this study contained 75, 91.2, 97.3, and 68.6 g/100 g of protein, respectively. When aliquots of these fractions were precipitated with 10 g/100 g of TCA, about 1.00, 0.93, 2.87, and 6.10 g/100 g of sugar were found in the total and major globulin, albumin, and glutelin fractions, respectively. The trypsin inhibitor activities in the chickpea flour and protein fractions are in **Table 3**. Data on the whole chickpea seed and unheated flour agree with the results of Sotelo et al. (*17*), who reported a variation from 9.0−15.7 TIU/mg of flour among nine Mexican varieties of

Table 2. Composition of Protein Fractions of Decorticated and Defatted Chickpea Flour

fraction	% protein	
	flour[a]	total protein
salt-soluble	17.55 ± 0.27	70.19 ± 1.10
albumins	4.66 ± 0.80	18.63 ± 3.19
total globulins	11.96 ± 0.93	47.85 ± 3.74
legumin-type globulin	10.63 ± 1.18	42.52 ± 4.72
prolamins	0.04 ± 0.01	0.17 ± 0.02
glutelins	2.50 ± 0.86	10.02 ± 3.43
insoluble	2.78 ± 0.22	11.14 ± 0.88
dialyzable[b]	1.65 ± 0.27	6.60 ± 1.07

[a] Decorticated and defatted chickpea flour, containing 25.01% of total protein. [b] Difference between salt-soluble proteins and the sum of albumins and globulins.

Table 3. Trypsin Inhibitor Activity in Chickpea Seeds and Protein Fractions

sample[a]	TIU[b]/mg of sample	TIU[b]/mg of protein	residual activity, after heat (%)
seeds	8.26 ± 0.12	46.88 ± 0.67	
unheated flour	9.68 ± 0.32	38.72 ± 1.29	
heated flour	0.53 ± 0.08	2.31 ± 0.38	5.97
unheated albumin	179.40 ± 0.57	239.20 ± 0.65	
heated albumin	4.10 ± 0.24	5.47 ± 0.35	2.29
unheated total globulin	8.63 ± 0.25	9.46 ± 0.28	
heated total globulin	1.41 ± 0.27	1.54 ± 0.29	16.28
unheated major globulin	5.18 ± 0.36	5.32 ± 0.36	
heated major globulin	0.43 ± 0.06	0.54 ± 0.06	10.15
unheated glutelin	3.14 ± 0.09	4.58 ± 0.13	
heated glutelin	0.0	0.0	0

[a] The heated samples were autoclaved at 121 °C for 15 min. [b] TIU, trypsin inhibitor unit. Values are means ± SD, $n = 3$.

chickpea. Attia et al. (*15*) reported values of 8.11 TIU/mg of whole seeds for chickpea Giza 1 cultivar. Unheated albumins showed by far the greatest values of TIU, as expected, because proteins with protease inhibition activities are concentrated in this fraction. As shown in **Table 3** these inhibitors were found to be heat labile in all fractions, where heating in steam (121 °C/15 min) significantly decreased the inhibitor activities. In this study those chickpea fractions that showed higher percentages of nonprotein compounds suffered a more efficient inactivation (**Table 3**). Although the mechanisms of this possible inactivation are not very well described, this fact has been observed by other authors in various materials (*18*).

Data on the amino acid contents in chickpea proteins are presented in **Table 4**. Aspartic and glutamic acids represented about 30 g/100 g of the amino acids in all chickpea proteins. Legume seed proteins are generally deficient in sulfur amino acids; however, contrary to other authors, Clemente et al. (*19*) have observed that methionine and cysteine were not limiting amino acids in chickpea seed proteins, relative to the FAO/WHO reference (*20*).

Thermal treatment of chickpea flour did not affect its digestibility, and the unheated and heated flours had apparent and true protein digestibilities significantly lower than those of the casein group (**Table 5**). Sotelo et al. (*17*) also found no significant differences between the apparent protein digestibility of raw and cooked chickpea for nine Mexican varieties. The unheated flour values were in accordance with results found by Khaleque et al. (*21*), who reported 76.11 and 78.38% for apparent and true protein digestibility, respectively. However, Nestares et al. (*7*) showed that cooking significantly improved the apparent protein digestibility of chickpea flour, regardless of different types of soaking solution used previously. El-Adawy (*22*) also found an increase below 7% in the in vitro digestibility of raw chickpea flour as a result of boiling, autoclaving, microwave cooking, and germination.

True digestibility of heated albumin, globulin, and major globulin did not differ significantly from that of supplemented casein as shown in **Table 5**. There was a great difference in the activities of trypsin inhibitor between albumin fractions, native and heated (**Table 3**), however; their digestibilities did not differ significantly. Moreover, it can be observed that, despite the trypsin inhibitor activity in unheated albumin samples being about 6 times higher than that in unheated chickpea flour (**Table 3**) and the digestibility of albumin was higher than that of the flour. This characteristic has been observed by other authors who found that a decrease in trypsin inhibitor activity in the chickpea albumin was not related to an increase in the in vitro digestibility (*19, 23*). Contrary to our results, various authors have found that cooking in water improved the apparent digestibility of chickpeas and indicate as one of the explanations for this effect the removal of trypsin inhibitor, showing a correlation between the TIU content and low protein digestibility in legumes (*7, 18, 24*). However, our results show that the heat did not influence the in vivo digestibility of the flour and albumin fraction, whereas the same was not observed with the globulin fraction, the in vivo digestibility of which showed an increase with heating (**Table 5**). This improvement in digestibility can be imputed to heat denaturation of the fraction, as already demonstrated by Carbonaro et al. (*25*) in studies involving proteins from chickpea, fava bean, dry bean, and lentil.

The results of this study show that heat denaturation of the major globulin (11S) from chickpea could play a major role in the increase of total globulin digestibility after heating, because Tavano and Neves (*16*) already have observed that the in vitro digestibility of the minor globulin fraction (7S) from chickpea was not altered by heating, whereas the major (11S) and total fractions suffered an improvement. In the case of chickpea globulins the heat denaturation might have exposed susceptible sites to proteolysis without the formation of resistant aggregates, contrary to observed for proteins from other sources as fava bean and lentil (*3, 25*).

Rubio et al. (*26*) observed similar results for in vivo digestibility of raw chickpea flour and globulin fraction. These observations suggest that the structural resistance of chickpea proteins to digestive enzymes, often imputed to globulins, would be not enough to explain the lower protein digestibility of chickpea flour, compared to casein. Moreover, the trypsin inhibitor activities in the samples also could not explain this observation. It is generally assumed that the presence of trypsin inhibitor and the slower digestion rate of legume proteins could explain their lower nutritional efficiency compared to animal protein. Nevertheless, this conclusion is usually based on experiments using whole seed meal in the diet. When we studied separate protein fractions, better results were observed than for the total chickpea protein in the flour, and similar results to the casein group were encountered when the fractions were heated, except for the heated glutelin sample (**Table 5**).

Rubio and Seiquer (*27*), who administered isolated globulins from chickpea (7S and 11S), fava bean, and lupin, in a liquid base (by gavage) to rats, found them to be highly digestible; however, when the protein fractions were incorporated in a solid diet, there was a decrease in their digestibility. The authors suggested that the un-denatured legume globulins are highly digestible in the small intestine and that the low protein digestibility is probably due to other factors present when whole flour is used. The glutelin fraction showed a low performance

Table 4. Amino Acid Compositions of Chickpea Proteins (Milligrams per 100 mg of Protein)[a]

amino acid	flour	albumin	total globulin	major globulin	glutelin	casein	FAO/WHO pattern		
							infant	child	adult
nonessential									
aspartic acid	12.28	12.92	13.42	12.95	12.15	6.2			
glutamic acid	18.03	15.75	19.59	19.40	17.12	18.6			
serine	5.55	5.26	6.08	6.09	5.39	5.0			
proline	4.25	3.97	4.32	4.40	4.12	8.1			
glycine	4.19	4.69	3.66	3.70	4.07	1.4			
alanine	4.48	4.93	3.92	4.03	4.69	2.5			
arginine	9.94	5.78	10.17	10.27	7.85	3.2			
essential									
threonine	3.93	5.21	2.95	2.88	4.35	3.8	4.3	3.4	0.9
valine	4.06	4.55	2.95	4.15	5.05	5.5	5.5	3.5	1.3
methionine	0.88	2.30	0.17	0.19	1.49	2.5			
cystine	1.07	2.45	0.82	0.68	0.38	1.0			
methionine + cystine	1.95	4.75	0.99	0.87	1.87	3.5	4.2	2.5	1.7
isoleucine	3.83	4.27	4.05	4.06	4.52	4.4	4.6	2.8	1.3
leucine	7.73	7.18	7.81	7.60	7.68	7.6	9.3	6.6	1.9
tyrosine	2.83	3.44	2.58	2.51	3.30	2.9			
phenylalanine	6.17	4.27	7.06	6.81	5.88	4.1			
tyrosine + phenylalanine	9.01	7.71	9.64	9.32	9.18	7.0	7.2	6.3	1.9
tryptophan	0.62	0.70	0.78	0.80	1.09	1.0	1.7	1.1	0.5
lysine	7.31	9.33	6.61	6.52	7.89	6.8	6.6	5.8	1.6
histidine	2.86	3.02	3.08	2.97	2.99	2.5	2.6	1.9	1.6

[a] Values underlined represent the first limiting amino acid of the sample.

Table 5. Apparent Digestibility (AD), True Digestibility (TD), Biological Value (BV), Protein Efficiency Ratio (PER), and Protein Digestibility-Corrected Amino Acid Score (PDCAAS) for Chickpea Flour and Protein Fractions[a]

diet[b]	in vivo protein digestibility		BV[c]	PER	PDCAAS	
	AD	TD[c]			estimated	casein (%)
CAS	93.82a ± 1.72	98.23a ± 1.43	95.66a ± 1.50	4.24a ± 0.37		
CASN	91.59a ± 1.67	96.08ad ± 1.61	92.67ac ± 3.32	2.48bd ± 0.63	87.34a ± 1.47	100
unheated flour	73.63b ± 7.90	78.42b ± 7.88	80.37bd ± 10.12	0.37c ± 0.44	43.91b ± 4.41	50.27
heated flour	74.13b ± 3.43	78.75b ± 3.42	87.58bc ± 7.67	2.88b ± 0.24	44.10d ± 1.92	50.49
unheated albumin	84.07cd ± 4.58	88.76ce ± 4.57	68.94de ± 6.41	2.05d ± 0.22	55.92c ± 2.88	64.02
heated albumin	88.49ac ± 5.04	93.00cd ± 5.04	82.03bf ± 1.99	2.32bd ± 0.64	58.59c ± 3.18	67.08
unheated total globulin	82.04d ± 2.46	87.05e ± 2.46	64.80e ± 11.87	2.12d ± 0.16	34.47d ± 0.67	39.47
heated total globulin	89.74a ± 2.18	94.61d ± 2.17	72.32def ± 12.08	1.96de ± 0.42	37.47e ± 0.86	42.90
heated major globulin	90.29a ± 2.27	94.65d ± 2.27	80.53bf ± 5.26	1.52e ± 0.28	32.56f ± 0.78	32.28
heated glutelin	73.70b ± 2.01	78.19b ± 2.01	47.53g ± 5.56	1.08f ± 0.29	58.49c ± 1.50	66.97

[a] Values are means ± SD, $n = 8$, and values in the same column with different letters (a−g) are significantly different ($p \leq 0.05$). [b] CAS, casein control, supplemented with L-Cys, 3 g); CASN, casein control, nonsupplemented). The heated samples were autoclaved at 121 °C for 15 min. [c] Average metabolic fecal nitrogen was 0.59 mg of N/g of intake diet, and average of metabolic urinary nitrogen was 1.2 mg of N/g of intake diet. Average weight loss of rats fed the protein-free diet was 11.70 g.

among the protein fractions in the rat assays (**Table 5**), in contradiction to the glutelin amino acid composition observed in **Table 4**, which was not as deficient in essential amino acids as the other fractions. This low nutritional performance of the glutelin fraction could be associated with the conditions of the extraction and also with its heating in the presence of a high concentration of carbohydrates associated with the fraction, as observed by Tavano (*28*).

The PER value for the supplemented casein sample were significantly higher than those for all of the chickpea samples; however, when casein diet was not supplemented, the values were similar to those of heated flour, unheated and heated albumin, and total globulin (**Table 5**). It can be observed that unheated flour showed very low values in rat growth assays, compared to its heated form. In this case, it is important to state that the rats fed unheated flour diets exhibited an increased refusal of this diet near the 10th day, starting a period of very low intake, which hindered the weight gain of these animals. The observation that the heated flour has not presented this effect to the animals could apparently indicate as a possible cause the presence of some heat-labile component in the flour.

Rubio et al. (*26*) observed that the in vivo digestibility, nitrogen retention, and NPU values obtained for chickpea flour and the globulin fraction were very similar, suggesting that the lower nutritional value of chickpea meal was due largely to poor utilization of the globulins in these seeds. Neves et al. (*29*) have also observed in lentil (var. Multolupa) that in vivo digestibility of the flour and the fractions were approximately that of casein, but that the incorporation of flour and fractions as sole protein source in the rat diets was not sufficient to sustain their growth at levels similar to those observed for supplemented casein diets.

Some authors give evidence that the thermal treatment does not cause a lot of alterations in chickpea amino acid composition (*7, 30*). To estimate PDCAAS, the same amino acid compositions found in the unheated chickpea samples (**Table 4**) were used for heated sample calculations as shown in **Table 5**. All chickpea values were much lower than the casein ones. Among chickpea fractions, the albumins and glutelins showed the best performances. Although globulin fractions have showed high digestibility, their very poor essential amino acid contents brought down the PDCAAS (**Table 5**), and these fractions had the lowest values.

Salgado et al. (*31*) concluded that white and black chickpea seeds seem to be satisfactory protein sources for weaned pigs, exhibiting little difference in their digestibility values or effects on the morphology and function of intestinal tissues. However, various authors working with purified lupin, fava bean, soybean, and chickpea proteins concluded that the nutritional performance of these legume meals may be related less to antinutritional factors than to the chemical structure of their major fraction, the globulins or the adverse effects of these proteins or their digestion products on nitrogen metabolism and on growth (*3, 25, 26, 28, 29*). In conclusion, the behavior of these proteins when isolated and in the raw and heated flour indicates that some other factors are involved, related both to the protein, such as molecular characteristics, interactions during passage through the gastrointestinal tract, and nitrogen metabolism, and to other components in the system that contribute to the excretion of endogenous nitrogen.

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