Evaluation of the chemical composition, protein quality and digestibility of lupin (*Lupinus albus* and *Lupinus angustifolius*)#

Avaliação da composição química, qualidade proteica e digestibilidade do tremoço (*Lupinus albus* e *Lupinus angustifolius*)

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Abstract

The purpose of this study was to determine the protein quality of lupin (*Lupinus albus* and *Lupinus angustifolius*) and to determine their chemical composition, including dietary fiber. The food transformation index, protein efficiency ratio and net protein ratio were significantly higher for the casein group than for the lupin groups. The in vivo digestibility value of casein was 95.81 ± 1.60, and the DV of *L. albus* and *L. angustifolius* were 90.89 ± 2.85 and 89.30 ± 2.01, respectively. Amino acid composition scores lower than one were found for almost all essential amino acids in lupin groups. The protein digestibility corrected amino acid scores ranged from 40.00% to 89.07% for *L. albus* and from 30.36% to 83.05% for *L. angustifolius*. It was concluded that the two varieties of lupins studied are good protein sources, with no statistical difference between them, and also that this protein has an excellent digestibility, besides being a good source of dietary fiber, especially *Lupinus angustifolius*. Therefore, the two varieties of lupine were considered as having good quality protein and as being a good alternative for human consumption.

Keywords: Lupinus. Food Composition. Biological Availability.

Resumo

A proposta deste trabalho foi determinar a qualidade proteica do tremoço (*Lupinus albus* e *Lupinus angustifolius*), além de determinar a sua composição centesimal, incluindo fibra alimentar. O Coeficiente de Eficiência Alimentar, o Quociente de Eficiência Proteica e o Quociente de Eficiência Líquida da Proteína foram significativamente maiores no grupo caseína que nos grupos experimentais. A Digestibilidade Verdadeira da caseína foi 95,81 ± 1,60, enquanto a de *L. albus* e *L. angustifolius* foi 90,89 ± 2,85 e 89,30 ± 2,01, respectivamente. Na análise da composição aminoacídica encontrou-se escores menores que 1 para quase todos os aminoácidos essenciais. O escore químico corrigido pela digestibilidade variou de 40,00% a 89,07% para *L. albus* e de 30,36% a 83,05% para *L. angustifolius*. Concluiu-se que as duas variedades de tremoço estudadas se apresentaram como uma boa fonte proteica, não havendo diferença estatística entre essas, e que essa proteína tem uma excelente digestibilidade, além de ser uma boa fonte de fibra alimentar, especialmente o *Lupinus angustifolius*. Portanto, as duas variedades de tremoço foram consideradas de boa qualidade proteica e uma boa alternativa para a alimentação humana.

Palavras-chave: Lupinus. Composição de Alimentos. Disponibilidade Biológica.

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INTRODUCTION

Legumes are very important for human consumption due to its high protein content. Due to its technological properties, their use has been carried out not only by consumption of whole grains, but also by its incorporation into products as flours, concentrates or isolates, in order to improve the stability, texture and nutritional aspect of preparations[1].

Among the various grain legumes studied, lupin is a source of vegetable protein with great potential for use in human food. These legume species are mainly grown in the Mediterranean and South America, and the most cultivated lupin species are *Lupinus albus* (white lupin) and *Lupinus angustifolius* (blue lupin). Lupin is cultivated for the following three main purposes: food for ruminants, grass to improve soil structure and human consumption. Flour has been reported to be used in the production of pasta, chips, breads and sausages, and the consumption of canned lupin seeds is common in European countries[2].

In Brazil, the lupine is a legume currently used for forage and animal feed, but it has great potential for human consumption. Besides owning about 40% protein is also considered a good source of lipids - mainly unsaturated fatty acids, dietary fiber - representing 40% of the weight of the grain, mineral and vitamins. Finally, the seeds of lupins has a good balance of essential amino acids[3-5]. In recent years, numerous human intervention studies have demonstrated that both protein and dietary fibre of lupin exert several physiological benefits[6,7].

Despite the high protein content of legumes, use of legumes as a source of protein is sometimes limited because of the low digestibility and nutritional quality of most vegetable proteins[8].

For lupin, however, studies have reported that the levels of undesirable constituents, such as phytic acid, alkaloids, saponins, lectins, trypsin inhibitors and protease inhibitors, which can affect protein digestibility, are low compared to soybeans and others legumes[4,9-11].

Given the great potential of lupin as a protein source and the lack of studies assessing the *in vivo* digestibility (DV) of lupin flour, this study aimed to evaluate the protein quality of two lupin species (*L. albus* and *L. angustifolius*) and determine the chemical composition.

METHOD

Genetic Material

The analysis was carried out on *L. angustifolius* var. IAPAR 24 and *L. albus* var. *Floresta* seeds, which were supplied by the Agronomy Institute of Paraná (IAPAR). The seeds were obtained by pooling samples from different vintages of the same species.

Preparation of Lupine Flour

To analyze the chemical composition of the seeds, 2 kg of seeds were ground (brand Tecnal) to give lupine flour. The material was ground and sieved to pass a 60 mesh sieve. The flour samples were packed in plastic containers and stored in a freezer at -18 °C, during one month, until that were utilized.

The grains used to produce the flour to be consumed in the experimental design were subjected to heat treatment in a conventional oven at 150 °C for 30 min as previously described by Carvalho[12]. After the heat treatment, the grains were ground in a food processor (Walita brand) and then cooled.

Determination of Chemical Composition

Protein concentration was determined by the semi-micro Kjeldahl method, and ash was quantified by incineration of the samples in a muffle. Total lipids were extracted with petroleum ether using a Soxhlet apparatus, and the moisture was quantified by drying the samples in a air circulation oven at 105 ± 1 oC. Total dietary fiber was quantified by the enzyme-gravimetric method. Analyses were performed as described by the AOAC13. Carbohydrates were estimated by difference. All analyses were carried out in triplicate.

Assessment of Protein Quality

Protein quality was evaluated by a biological assay lasting 14 days using 24 male

Wistar rats (*Rattus norvegicus*; *albinus* variety and *Rodentia* class). The rats were weaned at 23 days after birth, and they were obtained from the vivarium of the Center for Biological Sciences and Health at the Federal University of Viçosa (Viçosa-MG). The initial weight of the animals was 56.37 ± 4.94 g as recommended by the AOAC[13].

The animals were kept in individual stainless steel cages in a controlled environmental temperature of 22 ± 3 °C with a light cycle of 12 h, and the animals received food and water *ad libitum*. The body weight and food intake of the animals were recorded weekly throughout the experimental period.

The rats were divided into four experimental groups as follows: casein (standard), no protein; *L. albus* flour; and *L. angustifolius* flour. The rats were fed the experimental diets for 14 days.

Diet composition was based on the AIN-93G formula as follows: 9.5% protein, 7% fat, 1% vitamin mixture, 3.5% saline mixture, 13.2% dextrinated starch, 10% sucrose, 5% fiber, 0.3% L-cystine and 0.25% choline bitartrate[14].

For the evaluation of protein digestibility, animals were fed with diets labeled with 200 mg indigo carmine/100 g diet on the 8th and 11th day of the experiment. The marked feces were collected on the 9th day, and all feces were collected on the 10th and 11th day of the experiment. Moreover, the unmarked feces were collected on the 12th day of the experiment.

The collected feces were placed in individual containers and kept under refrigeration. The feces were later dried in an air circulation oven (Marconi brand) at 105 °C for 24 h. The feces were then cooled, weighed and ground by a mini processor (ARNO brand) to determine in triplicate the total nitrogen content using the semi-micro Kjeldahl method[13]. The nitrogen content of the feces from the experimental groups was used to calculate true digestibility[15].

The animals were euthanized at the end of the experiment by asphyxiation in a medium containing CO_2.

The protein efficiency ratio (PER) was calculated based on the weight gain of the test group compared to the protein intake of the test group according to the method described by Hegsted[16] modified for an experimental period of 14 days. The net protein ratio (NPR) was determined on 14th day of the experiment as the ratio between the weight gain of the test group (g) plus the weight loss of the no protein group (g) and the protein intake of the test group[15] as the formula below.

The food transformation index (FTI) was calculated as the ratio between the weight gain (g) and feed intake (g). The protein digestibility corrected amino acid score (PDCAAS) was calculated from the amino acid chemical score corrected by the lupin flour digestibility. For comparison, the suggested amino acid dose, as recommended by the Food and Agriculture Organization[17], for children who are two to five years old was used as a benchmark.

The procedures used in the biological assay adhered to the didactic / scientific practice of animal vivisection as approved by the Ethics Committee for Animal Experimentation (CETEA) at the Federal University of Minas Gerais (protocol n 023/07).

This study was conducted from February to December 2008.

Statistical Analysis

Statistical analysis (ANOVA) was carried out to determine the F-value. To determine the level of significance, Tukey's test at 5% probability was used for the comparison between means. Statistical analyses were performed using the SPSS statistical software package (version 3.0; 2003).

RESULTS AND DISCUSSION

Chemical Composition

Table 1 shows the chemical composition results of the lupin varieties analyzed.

The two lupin flour varieties (*L. albus* and *L. angustifolius*) had similar protein contents

(Table 1) corroborating with food composition studies and databases that have reported a protein content in the range of 30 to 40% for the lupin family[18-22].

Table 1. Chemical composition of lupin flours (*L. abus* and *L. angustifolius*), Belo Horizonte-MG, Brazil, 2013

Components	*L. albus* (g/100g flour)	*L. angustifolius* (g/100g flour)
Protein	36.30 ± 0.23	36.94 ± 0.36
Lipids	8.37 ± 1.21	4.39 ± 0.31
Ash	2.84 ± 0.05	2.64 ± 0.05
Moisture	7.45 ± 0.08	8.99 ± 0.16
Total Dietary Fiber	9.11 ± 2.74	23.41 ± 1.37
Carbohydrates*	35.93	23.63

Values expressed as means ± standard deviations.
* Carbohydrate values calculated by difference. Therefore, no standard deviation was included.

The protein levels in the *L. albus* and *L. angustifolius* flours were 36.30 and 36.94%, respectively. Erbas, et al[2] reported that *L. albus* flour contains a protein content ranging from 33 to 47%, and Mohamed, et al[9] obtained a 38% protein level for the same type of flour. Moreover, Volek and Marounek[23] reported a value closer to the value obtained in this study (36.30%) for *L. albus* flour. Lqari, et al[24] reported a protein content of 32% for *L. angustifolius* flour, and Pastor-Cavada, et al[11] reported a protein content of 26.6% for the same flour.

When comparing lupin to other sources of vegetable protein, both lupin varieties analyzed in this study had higher protein contents than lentils (23%) and beans (20%)[25], but they had lower protein contents than soybeans (values ranging from 40.4 to 44.07%)[26,27].

With regard to ash contents, the two lupin varieties had similar contents (Table 1). A previous study has reported that the fat content of lupin ranges from 6 to 13% highlighting a high concentration of polyunsaturated fatty acids[2]. In this study, *L. albus* was highlighted by lipid content (8.37 ± 1.21 g/100 g flour), which was similar to values reported by others[2,9,18,28].

Lupin flour also contains a high amount of dietary fiber, which has many desirable properties, such as a high water holding capacity (7.1 g/g fiber), and beneficial effects for human health[1]. In this study, the *L. angustifolius* flour had a higher amount of dietary fiber (23.41%) than the *L. albus* flour (9.11%).

Lqari, et al[24] reported a greater fiber amount in *L. angustifolius* flour than that found in the present study (23.41%) with a content corresponding to 37.6% of seed weight.

As for carbohydrates, the levels measured for both species (35.93% in *L. albus* and 23.63% in *L. angustifolius*) were superior to what has been previously reported for lupin[10,24,29]. Mohamed, et al[9] observed a carbohydrate content equivalent to 48% of the seed weight for *L. albus*, and Glencross, et al[30] reported a carbohydrate content of 43.8% for *L. angustifolius*. However, both of these groups analyzed total carbohydrate content without taking into account the amount of fiber.

King, et al[31] obtained results more similar to the results found in the present study. Excluding the starch content, these authors reported polysaccharide levels of 35.02% for *L. albus* and 40.02% for *L. angustifolius*.

Protein Quality

Weight gain, food consumption and FTI of the lupin groups were inferior to casein group (Table 2). *L. albus* flour led to a weight loss in rats by promoting a negative FTI. Animals fed with *L. angustifolius* flour had a small weight gain, but the weight gain was approximately 35 times less than the weight gain of the standard group. Food consumption in the test groups was approximately 2.5 times less than that of the casein group.

Table 2. Means and standard deviations of weight gain, feed consumption and food transformation index (FTI) of the groups receiving a diet based on casein or lupin (*L. albus* and *L. angustifolius*), Belo Horizonte-MG, Brazil, 2013

Groups	Weight gain (g)	Feed consumption (g)	FTI
Casein	56.67 [a] ± 10.19	163.32 [a] ± 19.55	0.35 [a] ± 0.03
L. albus	-0.83 [b] ± 2.32	60.43 [b] ± 7.08	-0.01 [b] ± 0.04
L. angustifolius	01.60 [b] ± 2.41	65.80 [b] ± 6.71	0.02 [b] ± 0.03

[a,b]. Means followed by same letter within the same column do not differ by Tukey's test at 5% probability.

Despite previous reports suggesting that lupin has few undesirable constituents[2,10,22], the low consumption of lupin observed in this study may be related to the presence of alkaloids in the experimental diets.

Although no significant difference in consumption between the two lupin species was found in this study, other studies have reported that *L. albus* has lower levels of alkaloids than other lupin species. However, this species also has higher levels of manganese, which can cause a loss of appetite in birds, pigs and sheep[22].

The animals that received lupin flour had quality protein indexes, such as PER, NPR and digestibility, lower than the indexes of the casein group. The average percentage of adequate casein, as measured by relative PER (R-PER), relative NPR (R-NPR) and relative digestibility (R-DV), ranged from 0.00 to 6.68%, 39.43 to 45.80%, and 94.86 to 93.20%, respectively (Tables 3 and 4).

Table 3. Means and standard deviations of the protein efficiency ratio (PER) and net protein ratio (NPR) of the casein-based diets and experimental diets, Belo Horizonte-MG, Brazil, 2013

Groups	PER	PER-R	NPR	NPR-R
Casein	3.69 [a] ± 0.35	100	4.42 [a] ± 0.30	100
L. albus	-0.14 [b] ± 0.36	0.00	1.74 [b] ± 0.42	39.43
L. angustifolius	0.25 [b] ± 0.38	6.68	2.02 [b] ± 0.32	45.80

[a,b]. Means followed by same letter within the same column do not differ by Tukey's test at 5% probability.

Table 4. In vivo protein intake, stool weight, nitrogen excretion, digestibility (DV) and relative digestibility (R-DV) of the groups fed with either a casein-based diet or experimental diets, Belo Horizonte-MG, Brazil, 2013

Groups	PTN intake(g)	Stool Weight (g)	Nitrogen excretion	DV	DV-R
Casein	15.29 [a] ± 1.83	03.14 [a] ± 0.77	0.35 [a] ± 0.11	95.81 [a] ± 1.60	100
L. albus	5.90 [b] ± 0.69	1.90 [b] ± 0.40	0.30 [b] ± 0.07	90.89 [b] ± 2.85	94.86
L. angustifolius	5.97 [b] ± 0.62	2.17 [b] ± 0.25	0.35 [b] ± 0.05	89.30 [b] ± 2.01	93.20

[a,b]. Means followed by same letter within the same column do not differ by Tukey's test at 5% *probability*.

According to Friedman and Gumbemann[32], protein sources with a PER less than 1.5, as was observed for the two lupin varieties in this study, are of low quality. Although the *L. angustifolius* flour had slightly better R-PER and R-NPR values than *L. albus* flour, the results indicated that the protein present in the lupin diets was not sufficient to cause weight gain or to maintain the body weight of the animals. Neves, et al[8] studied the flour and protein fractions of *L. albus,* and they also found low levels of NPR.

The relative PER and relative NPR values for lupin in this study were lower than those obtained by Pires, et al[33] for beans and soybeans. These authors reported R-PER and R-NPR values of 48.96 and 65.36%, respectively, for beans, and they also reported R-PER values of 39.06 and 40.35% and R-NPR values of 55.96 and 56.59% for the soybean varieties that they studied.

Table 4 shows that both lupin varieties had good digestibility, similar to the digestibility of cereals, such as rice (92.12%), oats (87.84%)[34] and wheat (89.44%), and both varieties had better digestibility than legumes, such as soybeans and beans, which have digestibility values ranging from 71.76 to 83%[27,33].

No data on the *in vivo* digestibility of lupin flour has previously been reported. However, Chew, et al[35] studied the *in vivo* digestibility of isolated protein from this legume, and they obtained a digestibility value of 98.2%. Others have reported the *in vitro* digestibility to be 86.9% for *L. albus*[36] and 86.3% for *L. angustifolius*[24]. These reported values were lower than the values obtained in the present study, except for isolated lupin protein, which was expected to have superior digestibility over lupin flour due to the extraction of various interfering factors.

Despite having lower protein contents than soybeans, lupin also has lower amounts of lectins, saponins and protease inhibitors[10]. Because these substances negatively interfere in protein digestibility, their presence in small amounts in lupin may explain the better digestibility of lupin when compared to beans and soybeans.

Contrary to the results reported by Van Barneveld[28] and Erbas, et al[2], Table 5 shows that the *L. albus* and *L. angustifolius* flours did not contain high concentrations of lysine.

Table 5. Amino acids, amino acid chemical score and PDCAAS of the lupin flours (*L. albus* and *L. angustifolius*) according to standards reported by the FAO / WHO[17], Belo Horizonte-MG, Brazil, 2013

Essential Amino acids	Aa per g of protein		Aa Score			PDCAAS	
	FT 1	FT 2	Standard FAO / WHO[17]	FT 1	FT 2	FT 1	FT 2
Phenylalanine + Tyrosine	43.45	31.27	46.00	0.94	0.68	85.44	60.72
Histidine	16.11	15.70	18.00	0.89	0.87	80.89	77.69
Isoleucine	20.30	15.43	31.00	0.65	0.50	59.08	44.65
Leucine	43.58	35.54	63.00	0.69	0.56	62.71	50.01
Lysine	29.10	25.90	52.00	0.56	0.50	50.90	44.65
Methionine + Cystine	13.94	09.37	26.00	0.54	0.36	49.08	32.15
Threonine	26.39	19.56	27.00	0.98	0.72	89.07	64.30
Tryptophan	9.34	06.89	7.40	1.26	0.93	-	83.05
Valine	18.27	14.46	42.00	0.44	0.34	40.00	30.36

FT 1 represents *L. albus* flour. FT 2 represents *L. angustifolius* flour.
Amino acid score = Aa per grams of PTN / standard; PDCAAS = Aa limiting x digestibility of *in vivo* experiment (DV of *L. albus* = 90.89; and DV of *L. angustifolius* = 89.30).

The evaluation of the lupin amino acid composition demonstrated that there was a deficiency of all essential amino acids, except tryptophan in *L. albus*, for both lupin species. Lqari, et al[24] analyzed *L. angustifolius* flour and found deficiencies in lysine, histidine, tyrosine and methionine. Pastor-Cavada, et al[11] also observed deficits of valine and tryptophan. Despite the good digestibility when compared to other legumes, the studied lupin species had lower protein quality in relation to the presence of essential amino acids.

CONCLUSION

The two species of lupine surveyed in this study had levels similar to high protein reported for soy protein. With the digestibility of protein, lupine proteins were superior to other legumes, such as soy and beans.

Both varieties showed a high lupine protein and that had a high digestibility, despite the values of PER, NPR and NPU were lower than those obtained by casein. No statistical difference between the two species of lupine in relation to protein quality indexes evaluated in this research.

Regarding the chemical composition evaluated was no statistical difference between the two varieties only to the amount of fiber in the *Lupinus angustifolius* was higher to *Lupinus albus*.

The lupine would be a good alternative source of protein, allowing the nutritional enrichment of foods and making them economically viable for underserved populations. Thus, it creates great potential for use in the food industry.

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