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Effect of Aqueous, Acid, and Alkaline Thermal Treatments on Antinutritional Factors Content and Protein Quality in *Lupinus campestris* Seed Flour

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Lupinus campestris seeds grown in Mexico have a similar composition to soybean (44% protein, 13% lipids). Use of lupin seed in human and animal diets is limited by its quinolizidine alkaloids (QAs) and other antinutritional factor contents. The effect of aqueous, acid, and alkaline thermal debittering treatments with *L. campestris* seeds flour was evaluated on QAs, oligosaccharides (RFOs), and phenolic compounds (PCs) contents, as well as protein quality. The alkaline treatment most effectively eliminated QAs. Protein content increased from 430 g/kg in untreated seeds to 543 in the aqueous treatment, 567 in the alkaline treatment, and 563 g/kg in the acid treatment. RFOs were eliminated in all treatments. The obtained sample with alkaline treatment had the best protein quality (2.04); this value was 17% lower than that of casein (2.45). The apparent digestibility was over 90% in all treatments, with the aqueous treatment exhibiting the best value (93%).

KEYWORDS: ***Lupinus*; quinolizidine alkaloids; oligosaccharides; protein quality; legumes**

INTRODUCTION

Legumes account for about 20% of worldwide protein intake in human diets. Native to the Mediterranean and the Americas, lupin species have been cultivated for centuries as animal and human food. Their seeds are one of the richest plant protein sources, and nutritional studies in animals and humans have shown that *Lupinus* genus (*Fabaceae*) species have protein content comparable to that of soy bean (*Glycine max*). Protein content and amino acid profile vary between lupin species, but intraspecies variability is low. Like other lupin species, *Lupinus campestris* seed has high protein content (280−430 g/kg). Broader use of lupin as a protein source is limited by its high content of quinolizidine alkaloids and other antinutritional factors. These bitter compounds make the seed unpalatable and sometimes toxic; indeed, some alkaloids cause neuromuscular blockage, respiratory depression, cyanosis, cramps, and cardiac arrest at toxic doses (*1*). It is therefore vital to know both the quantity of alkaloids in a given source and the toxicity of individual alkaloids, since they do not all have the same activity. Treatments such as cooking, soaking, germination, and fermentation are known to eliminate several antinutritional factors, including QAs, and to improve legume nutritional value, transforming it into a protein-rich food (*2*, *3*).

Protein nutritional quality is a function of the concentration, availability, and proportions of essential and nonessential amino acids in a source, sufficient levels of which allow optimum utilization (*4*). Amino acid availability varies by protein source, type of cooking treatment, and interaction with other dietary components. Deficiency in one or more essential amino acids is considered a mark of low protein quality. Bioassays are applied to measure the efficient use of biological protein as an amino acid source under controlled conditions (*5*). Many bioassay methods are based on the biological effects of protein quality on animal growth. These assays include parameters such as protein efficiency ratio (PER) based on weight gain and net protein ratio (NPR) determined by inclusion of animals fed a protein-free diet.

The objective of the present study was to determine the effect of aqueous, alkaline, and acid thermal debittering treatments of *Lupinus campestris* seed on quinolizidine alkaloids (QAs), oligosaccharides (RFOs), and phenolic compounds (PCs) contents and protein quality parameters, such as protein efficiency ratio, corrected protein efficiency ratio, net protein ratio, and true and apparent digestibility.

MATERIAL AND METHODS

Wild *L. campestris* seeds were collected along a 50 km section of the Oaxtepec-Xochimilco highway in Morelos state, Mexico.

Chemical Reagents. Methanol; ethanol; acetonitrile; deionized water; sucrose, melibiose, raffinose, stachyose, and verbascose standards;

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trichloroacetic acid; dichloromethane; and other chemical reagents were purchased from Sigma Chemical Co. (St Louis, MO). Lupanine was donated by Dr. Mercedes Muzquiz of the INIA, Madrid, Spain, and Ciceritol was supplied by Dr. Gulewicz of the Institute of Bioorganic Chemistry, PAS.

Debittering Seeds. Seeds were cleaned of foreign material (e.g., vegetal remains, stones) and any immature or damaged seeds. Three batches of seeds were hydrated with one of the debittering solutions—water, $NaHCO_3$ (0.5% w/v, pH 11.4), or citric acid (0.1% w/v, pH 2.72) at 1:10 w/v—and heated to boiling (93 °C) for 6 h. The water or each solution, depending of treatment, was changed every 20 min.

Chemical Analyses. Protein (NX5.7, method 955.04), lipids (method 920.39), total dietary fiber (method 985.29), and ash (method 923.03) were determined according to AOAC standard methods (*6*).

Quinolizidine Alkaloids Extraction and Quantification. QAs were extracted as described by Muzquiz et al. (*7*). Finely ground lupin seeds (0.5 g) were homogenized three times with 5 mL of 5% trichloroacetic acid with an Ultra Turrax (Staufen, Germany) and centrifuged at 10 000*g*/10 min at 4 °C. The supernatant was treated with 0.8 mL of 10 M NaOH to change alkaloids into free bases and then extracted three times with 15 mL of dichloromethane. The dichloromethane extracts were combined and evaporated at 30 °C until dryness. The residue was dissolved in 1 mL of methanol, and a codeine solution as an internal standard (final concentration of codeine, 1 mg/mL) was added.

Quinolizidine alkaloids (QAs) were quantified by GC/MS using a Perkin-Elmer Chromatography Autosystem (Walthama, MA), equipped with a phosphorus−nitrogen detector (PND) and using the Turbochrom program (Waltham, MA) for instrument control and data analysis. A SPB-1 column (30 m × 0.25 mm i.d.) was used with helium as the carrier gas (1.38 bar). The injector temperature was 240 °C, and the detector temperature was 300 °C. The initial oven temperature was 150 °C; it was raised 5 °C/min to 235 °C and left at this final temperature for 23 min. A calibration curve was generated for lupanine with a linear response within a 0.25−1.25 mg/mL range with the determination coefficient (r^2) = 0.99

Capillary CGMS was applied for alkaloids identification. A Perkin−Elmer Autosystem XL gas chromatograph (using the same column and conditions as above) was coupled to a mass selective detector (Perkin−Elmer Turbomass Gold), running the Turbomass software.

Carbohydrates Extraction and Quantification. Carbohydrates (CHs) were extracted from *L. campestris* samples, following Muzquiz et al. (*8*), with some modifications. Briefly, seeds (0.1 g) were ground and then homogenized with 5 mL of aqueous ethanol (50%) for 1 min at 4 °C. The mixture was centrifuged for 5 min (3020 g) and the supernatant recovered. This procedure was repeated twice and the combined supernatants passed through a Waters Sep-Pak, Vac 6 cm^3 (C-18 at 500 mg) with a Supelco vacuum system (St Louis, MO). The extract was evaporated in a vacuum and the residue dissolved in deionized water (1 mL). This was centrifuged at 1210*g* for 10 min, and the supernatant filtered through a 45 μm filter.

Samples (20 μL) were analyzed using a Beckman HPLC chromatograph 156 refractive index detector (Ramsey, MN). A Spherisob-5-NH2 column (250 mm × 4.6 mm i.d.) (Milford, MA) was used with acetonitrile/water (65:35, v:v) as the mobile phase at a flow rate of 1 mL/min. Individual sugars were quantified by comparison with external sucrose, melibiose, raffinose, ciceritol, and stachyose standards. Calibration curves were prepared for all sugars, with a linear response in the 1−5 mg/mL range and determination coefficient (r^2) = 0.99.

Phenolic Compounds Analysis. Quantitative analysis of phenolic compounds (PCs) was done by spectrophotometry (*6*), using Folin-Dennis reagent and a Na_2CO_3 solution. Extraction was done with a methanol/water (50:50) solution, and tannic acid (1−10 mg/mL) was used to prepare the standard curve. Readings were taken at 760 nm, with values exhibiting linear behavior and using a determination coefficient (r^2) = 0.99. All results were expressed as the average of three replicates ± standard error.

Protein Quality. The protein quality of the reference (casein) and proteins from the three debittering treatments was evaluated in 40 growing Wistar rats (42.5 ± 5.8 g initial weight). Each protein diet was tested on 10 randomly selected animals housed in individual cages under controlled conditions (20 ± 2 °C; 55% relative humidity; 12 h light/12 h dark cycle). The diet composition was 10 g of protein; 10 g of corn oil; 4 g of mineral mixture; 1 g of vitamin mix; 5 g of cellulose; and 70 g of corn starch per 100 g (902.3 method AOAC (*6*)). Vitamin (AIN-93-VX) and mineral (AIN-936-MX) mixes were obtained from Harland Teckland Laboratory Animal Diets (Madison, WI). Casein was used as a reference. Food and water were provided *ad libitum*. The protein efficiency ratio (PER) was calculated by feeding the rats with the test diets for 28 days and by measuring feed intake daily and weight gain weekly. The net protein ratio (NPR) was evaluated over a 14-day period by feeding a separate group of 10 animals with a protein-free diet for 14 days, measuring endogenous nitrogen uptake and average weight loss, and calculating the NPR. Apparent digestibility (AD) and true digestibility (TD) were determined according to Eggun (*9*). In the animal group fed the protein-free diet, TD was corrected for endogenous nitrogen feces excretion. Protein digestibility was calculated by collecting feces between days 14 and 21 of the assay period, and then drying, weighing, milling, and storing it until protein determination. Feed and fecal nitrogen contents were analyzed by the Kjeldahl method (955.04; AOAC (*6*)).

Statistical Analysis. All results were analyzed using a one-way analysis of variance (ANOVA) and a Tukey's test to compare means. The significance level was defined as $\alpha \leq 0.05$.

Table 1. Chemical Composition of Untreated *Lupinus campestris* Seed Flour before and after Debittering with Aqueous, Alkaline, and Acid Thermal Treatments (g/kg of seed)[a]

component	untreated	treatments		
		aqueous	alkaline	acid
protein (NX5.7)	397 ± 2.33	497 ± 4.60	517 ± 2.91	513 ± 1.02
lipids	108 ± 2.52	132 ± 0.31	158 ± 1.40	128 ± 0.91
fiber	147 ± 1.01	121 ± 1.53	105 ± 1.10	108 ± 1.63
carbohydrates	316 ± 2.80	226 ± 5.90	194 ± 5.94	220 ± 0.64
ash	32 ± 1.03	24 ± 0.70	26 ± 0.20	31 ± 0.63

[a] Results are the average of three replicates ± SD.

RESULTS AND DISCUSSION

The protein content of the untreated raw *Lupinus campestris* seeds was 397 g/kg (**Table 1**), which is similar to that of other lupin species (*3*) and soybean (*4*). The lipid content (108 g/kg) was similar to that of *L. albus* seeds, higher than that of *L. luteus* and *L. angustifolius* seeds (45−86 g/kg) (*10*), and lower than that obtained for *L. mutabilis* seeds (170−210 g/kg) (*11*). The total dietary fiber content of the untreated raw *Lupinus campestris* seeds (147 g/kg) was similar to that of *L. angustifolius*, *L. luteus*, and *L. albus* seeds (*10*).

The debittering treatments produced changes in the *L. campestris* seeds protein composition (**Table 1**). Protein content increased from 397 g/kg in the untreated seeds to 497, 517, and 513 g/kg in seeds in the aqueous, alkaline, and acidic thermal treatments, respectively. This increase is probably due to reductions corresponding to solubilization of carbohydrates, minerals, and total dietary fiber contents during each treatment. The alkaline treatment was most efficient for eliminated CHs, followed by the acidic and aqueous treatments (*12*).

Quinolizidine Alkaloids Content. Lupin alkaloids can be eliminated by technological treatments of bitter seeds. In the untreated *L. campestris* seed, QAs content was 2.34 g/100 g (**Table 2**), confirming that QAs content is a major obstacle to use of untreated *L. campestris* seed in human or animal diets. This content is similar to that of *L. albus* (2.36 g/100 g) and other *L. campestris* varieties (2.46 g/100 g) (*13*). It is higher than the QAs range reported by Boschin et al. (*14*) for *L. albus* (0.005−0.056 g/100) but within the range reported for *L. angustifolius* (0.0141−0.0701 g/100 g). QAs content gives food or feed a bitter taste, limiting intake. Bitterness varies by alkaloid type (*15*), and QAs concentration and type varies between species. Within species, location and climate can cause variations in relative alkaloids proportion between cultivars (*1*). Lupin variety type (i.e., bitter or sweet) is determined by its QAs content. Alkaloids

Table 2. Quinolizidine Alkaloids Contents in Untreated *L. campestris* Seed Flour before and after Aqueous, Alkaline, and Acid Thermal Treatments (g/100 g)[a]

treatment	α-isolupanine	5,6-cehydro-lupanine	lupanine	hydroxy aphylline	hydroxy aphyllydine	multiflorine	epi-hydroxy aphylline	dehydroephydroxy-aphylline	total	% elimination
untreated	0.002	0.049	0.002	0.499	1.678 ± 0.01	0.005	0.037	0.065	2.34 ± 0.06	0
aqueous										
1 h	0.001	0.012	ND	0.243 ± 0.01	1.592 ± 0.01	0.003	0.005	0.032	1.89 ± 0.01	19
3 h	ND	0.007	ND	0.170	1.279 ± 0.03	ND	ND	0.034	1.45 ± 0.03	38
6 h	ND	ND	ND	0.045	0.214 ± 0.01	ND	ND	ND	0.26 ± 0.07	89
acidic										
1 h	0.001	0.019	ND	0.249	1.595 ± 0.01	0.004	0.014	0.039	1.92 ± 0.01	18
3 h	ND	0.019	ND	0.226 ± 0.01	1.020 ± 0.04	ND	0.023	0.054	1.37 ± 0.06	42
6 h	ND	ND	ND	0.102 ± 0.03	0.181 ± 0.00	ND	ND	ND	0.28 ± 0.03	88
alkaline										
1 h	ND	0.01	ND	0.275 ± 0.01	1.505 ± 0.03	0.002	0.023 ± 0.05	0.043	1.86 ± 0.01	20
3 h	ND	0.010	ND	0.206 ± 0.06	1.180 ± 0.02	ND	0.017	0.028	1.44 ± 0.01	38
6 h	ND	ND	ND	0.047	0.073 ± 0.01	ND	ND	ND	0.120	95

[a] Results are the mean of two replicates ± SD. Where SD is not shown, the values were smaller than 0.00. ND = not detected.

content in some yellow lupin species exceeds that of the blue and white lupins examined, varying between 0.5 and 2.4 g/100 g dry matter, which are higher than the recommended levels. Godfrey et al. (*16*) and Sujak et al. (*10*) state that the alkaloids content in fodder and protein concentrates should not exceed 0.02−0.04%, since high alkaloids content may cause decreased digestibility in addition to neurological disorders such as convulsions, dizziness, and disturbances in breathing.

Of the total QAs in the raw seed, hydroxyaphyllidine accounted for 72% and hydroxyaphilline for 21% (**Table 2**), which is similar to previously reported levels (*17*). The remaining 7% included lupanine, 5,6-dehydrolupanine, multiflorine, epihydroxyaphyilline, and dehydroepihydroxyaphylline. These were tentatively differentiated by their characteristic ions observed with mass spectrometry and comparison with previously reported data (*13*). In species such as *L. albus*, lupanine is the most abundant alkaloid, while in *L. angustifolius*, lupanine and angustifoline are the majority alkaloids (*13*).

After debittering, QAs content decreased by 89% in the aqueous thermal treatment (0.26 g/100 g), 88% in the acidic treatment (0.28 g/100 g), and 95% in the alkaline treatment (0.12 g/100 g). These results are consistent with previously reported reduction levels; for instance, Torres et al. (*18*) reported a 98.6% reduction in total QAs content with aqueous and alkaline thermal treatments.

Debittering also reduced the original hydroxyaphylline and hydroxyaphyllidine concentrations (0.49 and 1.68 g/100 g) to 0.045 and 0.214 g/kg in the aqueous treatment, 0.102 and 0.181 g/100 g in the acidic treatment, and 0.047 and 0.073 g/100 g in the alkaline treatment.

The final QAs content was 0.26 g/100 g after the aqueous treatment, 0.28 g/100 g after the acidic treatment, and 0.12 g/100 g after the alkaline treatment. Although substantial reductions were attained in the present study, the final QAs contents in all the treatments were still above safe limits (<0.04 g/100 g) for human and animal consumption (*13*, *14*). Levels above 0.05 g/100 g can produce decreased food intake and a consequent drastic slowdown in animal growth. Among domestic animals, rabbits are most tolerant of QAs, since they can consume rations containing up to 50% lupin flour, acquire all necessary protein, and experience only slight decreases in growth. Other animals are more affected by QAs type than overall quantity; for example, sparteine is very toxic to pigs (*15*). This QA is not present in *L. campestris* seeds.

Very little research has been done on chronic QAs toxicity. It is generally assumed that these alkaloids are water-soluble, meaning an organism can easily eliminate them and therefore prevent any cumulative toxic effect. A study of rats fed *L. albus* flour (0.025% lupanine) for two generations showed no harmful effects. In addition, rats which survive a lethal dose recover completely, manifest no signs of clinical abnormality, and subsequently attain normal weight and physiological maturity comparable to those of untreated rats. Apparently, intake of lupin seeds with some alkaloid content does not produce serious danger to organism survival, although toxicity tests must be done to ensure safety, particularly when large quantities are involved (*15*).

Carbohydrate Content. The total carbohydrate (CHs) content in the unprocessed *L. campestris* seed was 12.03 g/100 g and consisted mainly of mono-, di-, and oligosaccharides (**Table 3**). The sucrose concentration was 2.14 g/100 g. Martinez Villaluenga et al. (*20*) reported considerable variation in sucrose content between species: *L. angustifolius*, 2.9−5.0 g/100 g; *L. albus*, 2.2−3.1 g/100 g; *L. luteus*, 1.0−1.4 g/100 g.

Total RFOs concentration in the untreated *L. campestris* seed was 9.02 g/100 g. Stachyose was the most abundant (5.72 g/100 g) of the RFOs, with levels similar to those reported for *L. angustifolius*, *L. albus*, and *L. luteus* (3.62−8.61 g/100 g) (*19*), soybean (4.8−5.97 g/100 g) (*20*), and cowpea (2.52 g/100 g) (*21*). Melibiose and ciceritol were also identified at lower concentrations (0.58 and 0.48 g/100 g, respectively). Differences in CHs content may be due to species, variety, climatic conditions, and soil, although determination methods can also affect recorded values (*8*). All three debittering treatments decreased RFOs content (**Table 3**), suggesting these compounds were solubilized. Decreases in RFOs content are important because these compounds produce flatulence in monogastric animals (e.g., humans) due to a lack of the α-galactosidase needed to cleave the glycosidic bond in these sugars (*22*). The carbohydrates elimination rate achieved with the three debittering treatments was up to 97%, higher than that reported for other legume treatments (*21*, *22*).

Phenolic Compounds. The phenolic compounds (PCs) content in the unprocessed *L. campestris* seed was 0.51 mg of tannic acid equiv/100 g (**Table 4**), which is considerably higher than the 0.15 and 0.223 mg/100 g reported for soybean and cowpea, respectively (*23*).

Phenolic compounds (PCs) content was reduced by 65% with the aqueous treatment, 67% in the alkaline treatment, and 76% in the acidic thermal treatment. These results agree with those of Mubarak (*24*), who found that different treatments reduced PCs content in mung bean seeds (*Phaseolus aureus* L.) by 39.4 to 62.1%. In cowpea seeds, Udensi et al. (*25*) reported that PCs were reduced 37% by boiling in water for 45 min, 12% after roasting at 120 °C for 30 min, and 37% after autoclaving at 120 °C/15 min. The present results also coincide with those of Rehman and

Table 3. Sucrose, Melibiose, Ciceritol, and RFOs Contents in Untreated *L. campestris* Seed Flour before and after Aqueous, Alkaline, and Acid Thermal Treatments (g/100 g)[a]

treatment	sucrose	melibiose	ciceritol	RFOs raffinose	RFOs stachyose	RFOs verbascose	total RFOs	% elimination
untreated	2.14 ± 0.13	0.58 ± 0.14	0.48 ± 0.13	1.16 ± 0.15	5.72 ± 0.16	1.94 ± 0.18	8.82 ± 0.06	0
aqueous								
1 h	1.99 ± 0.06	0.22 ± 0.09	0.54 ± 0.00	0.85 ± 0.10	4.85 ± 0.01	1.64 ± 0.01	7.34 ± 0.09	17
3 h	1.68 ± 0.17	0.20 ± 0.65	0.35 ± 0.02	0.76 ± 0.03	4.49 ± 0.30	1.54 ± 0.10	6.79 ± 0.11	23
6 h	0.07 ± 0.02	ND	ND	0.11 ± 0.00	0.29 ± 0.06	ND	0.40 ± 0.06	96
acidic								
1 h	2.11 ± 0.08	0.47 ± 0.23	0.54 ± 0.27	1.23 ± 0.67	5.71 ± 0.01	1.84 ± 0.06	8.78 ± 0.11	0.5
3 h	1.77 ± 0.07	0.32 ± 0.05	0.27 ± 0.01	0.97 ± 0.05	4.55 ± 0.04	1.50 ± 0.06	7.02 ± 0.26	20
6 h	0.15 ± 0.01	ND	ND	0.12 ± 0.01	0.54 ± 0.05	ND	0.66 ± 0.015	93
alkaline								
1 h	2.12 ± 0.05	0.40 ± 0.02	0.53 ± 0.01	1.27 ± 0.04	5.50 ± 0.08	1.86 ± 0.07	8.63 ± 0.03	2
3 h	1.40 ± 0.13	ND	0.30 ± 0.05	0.81 ± 0.08	4.10 ± 0.22	1.28 ± 0.09	6.19 ± 0.57	30
6 h	0.06 ± 0.00	ND	ND	ND	0.32 ± 0.08	ND	0.32 ± 0.08	96

[a] Results are the mean of three replicates ± S.D. ND = not detected.

Table 4. Phenolic Compounds Content in Untreated *L. campestris* Seed Flour before and after Aqueous, Alkaline, and Acid Thermal Treatments (mg of tannic acid equiv/100 g)[a]

time (h)	treatment: aqueous	acid	alkaline
0	0.51 ± 0.01	0.51 ± 0.009	0.51 ± 0.01
1	0.49 ± 0.00	0.50 ± 0.02	0.48 ± 0.01
3	0.37 ± 0.02	0.47 ± 0.01	0.36 ± 0.01
6	0.18 ± 0.01	0.12 ± 0.01	0.17 ± 0.00

[a] Results are the average of three replicates ± SD.

Shah (*26*), who stated that tannin content in black, red kidney, and white kidney beans was significantly reduced after ordinary cooking and pressure cooking at 121 °C for 20 min. Of the three tested debittering treatments, the acidic thermal treatment was most effective at lowering PCs content, producing a reduction 9% greater than the other treatments (**Table 4**).

Biological Quality of *L. campestris* Protein. In each treatment, the highest reductions in QAs, RFOs, and PCs content were produced after 6 h; therefore, protein quality in each treatment was assessed with samples treated for this amount of time.

***In Vivo* Digestibility.** Feed intake and weight gain did not differ ($p > 0.05$) between the three tested diets containing debittered *L. campestris* seeds, but all were lower ($p < 0.05$) than those for the control diet (**Table 5**). Feed conversion efficiency (FCE) was consequently highest in the control treatment. The average weight gain in the three debittered treatments was lower than the 2.75 g/day reported in rats fed a diet containing *L. albus* and the 3.09 g/day in rats fed a diet containing *L. luteus* (*27*); however, the feed intake in this study was almost twice that recorded in the present study, meaning the FCE observed here for debittered *L. campestris* flour is equal to that for *L. luteus*.

The protein efficiency ratio (PER) was highest in the casein treatment (2.45), although this did not differ ($p > 0.05$) from the alkaline (2.04) and acidic (1.83) treatments (**Table 6**). The acidic and alkaline treatments had PER values within the 1.80−2.48 range reported for wild legumes seeds from the Sonoran Desert (*28*), and they are also comparable to PERs reported for soybean (1.95), peas (1.89), beans (2.14), and chickpea (1.69) (*29*). Of the three experimental diets, the alkaline treatment had the best PER value. This treatment most effectively removed QAs and oligosaccharides, and it may therefore have improved feed palatability relative to the other diets due to its lower alkaloid concentration and consequent better sensory characteristics (*15*).

Table 5. Weight Gain, Feed Intake, and Feed Conversion Efficiency (FCE) in Rats Fed Diets Containing *L. campestris* Seed Flour after Aqueous, Acidic, and Alkaline Thermal Treatments during a Three-Week Trial[a]

treatment	weight gain (g/day)	feed intake (g/day)	FCE (weight gain/g feed intake)
casein	1.81 ± 0.24 [a]	7.09 ± 0.48 [a]	0.26 ± 0.02
aqueous	1.06 ± 0.09 [b]	6.02 ± 0.27 [b]	0.17 ± 0.02
acid	1.12 ± 0.13 [b]	6.02 ± 0.27 [b]	0.18 ± 0.02
alkaline	1.26 ± 0.14 [b]	6.09 ± 0.48 [b]	0.21 ± 0.01

[a] Means ± standard deviation of ten animals. Means in the same column with different letters are significantly different ($p < 0.05$).

Table 6. Total Weight Gain, Protein Efficiency Ratio (PER), and Corrected Protein Efficiency Ratio (cPER) in Rats Fed Diets Containing *L. campestris* Seed Flour after Aqueous, Acid, and Alkaline Thermal Treatments during a Three-Week Trial[a]

treatment	weight gain (g)	PER	cPER
casein	38.01 ± 5.17 [a]	2.45 ± 0.20 [a]	
aqueous	19.73 ± 3.15 [b]	1.48 ± 0.21 [b]	1.51 ± 0.31 [a]
acid	22.61 ± 1.69 [b]	1.83 ± 0.21 [a]	1.87 ± 0.20 [a]
alkaline	24.46 ± 3.06 [b]	2.04 ± 0.24 [a]	2.08 ± 0.23 [a]

[a] Means ± standard deviation of ten animals. Means in the same column with different letters are significantly different ($p < 0.05$).

Table 7. Net Protein Ratio (NPR), Apparent Digestibility (AD), and True Digestibility (TD) of Casein and *L. campestris* Seed Flour after Aqueous, Acidic, and Alkaline Thermal Treatments[a]

treatment	NPR	AD	TD
casein	3.90 ± 0.52 [a]	92.37 ± 0.98 [a]	94.30 ± 0.83 [a]
aqueous	2.81 ± 0.50 [a,b]	92.90 ± 0.83 [a]	93.83 ± 0.90 [a]
acid	3.33 ± 0.45 [b]	91.10 ± 1.11 [a]	91.97 ± 1.12 [a]
alkaline	3.62 ± 0.52 [a,b]	92.56 ± 1.74 [a]	93.32 ± 1.46 [a]

[a] Means ± standard deviation of ten animals. Means in the same column with different letters are significantly different ($p < 0.05$).

The NPR values did not differ ($p > 0.05$) between the three treatments containing debittered lupin meal (**Table 7**). The NPR values were higher than PER values because NPR measures protein efficiency based on animal growth and maintenance needs, allowing evaluation of proteins which do not promote growth. The PER value, in contrast, considers only growth needs, meaning that even if a diet has a PER of zero, it can still meet maintenance needs. This value can therefore underestimate protein quality in many cases.

Digestibility indicates the amount of protein nitrogen absorbed. True and apparent digestibility (**Table 7**) in the three treatments containing debittered *L. campestris* meal seeds did not differ ($p > 0.05$) from the control. The values observed in the present study were higher than the 85−90% reported by Donovan et al. (*30*) for two sweet lupin species (*L. albus*) with added 0.2% L-methionine, which in turn was similar to the digestibilities for soybeans under the same conditions. Addition of methionine to beans (variety Carioca 80) increases digestibility values from 64.1 to 65.2 (*31*).

Overall, the *in vivo* results indicate that the protein from debittered *L. campestris* seed has good nutritional value. This places *L. campestris* protein among the vegetable proteins with good digestibility, although the values are still lower than those for animal proteins.

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