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Improvement in food intake and nutritive utilization of protein from *Lupinus albus* var. *multolupa* protein isolates supplemented with ascorbic acid

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Abstract

The protein quality of protein isolates from lupin (LPI) (*Lupinus albus* var. *multolupa*), prepared by isoelectric precipitation, was assessed by chemical analysis of protein and amino acids and biological analysis of digestive and metabolic utilization of protein by growing rats. The animals were fed isonitrogenous and isocaloric diets adjusted to meet their nutrient requirements, in which lupin protein isolate was the only protein source, complemented with 0.5% methionine. Different LPIs were prepared with addition, or not, of ascorbic acid as antioxidant. Protein isolates had a protein content of 87.8–98.1%. Manganese content of protein isolates was reduced by 72.8–89.5% compared to the raw seed flour. Results from *in vivo* experiments showed that addition of 0.5% ascorbic acid to LPI incorporated into diets led to a 82.8% increase in daily food intake, when compared to the non-supplemented LPI, reaching similar values to those obtained with a casein–methionine control diet. Digestive and metabolic utilization of protein from LPI, assessed by nitrogen absorption or apparent digestibility coefficient, and by nitrogen balance or percentage of retained to absorbed nitrogen, respectively, was high, when the dietary intake of animals fed the LPI diets was adequate after addition of 0.5% ascorbic acid, although slightly inferior to the values obtained with a casein–methionine control diet. The high nutritive utilization of protein was reflected in excellent growth and nutritional indices assayed. In conclusion, ascorbic acid supplementation led to an improvement in the palatability of the LPI diets and, therefore, in daily food intake, which was reflected in a higher nutritive utilization of protein and improvement in weight gain and the food transformation index.

Keywords: *Lupinus albus* var. *multolupa*; Protein isolates; Ascorbic acid supplementation; Biological evaluation; Growing rats

1. Introduction

Due to the large amounts of saturated fat and cholesterol usually present in animal protein sources, most health organizations recommend the frequent consumption of vegetable protein, which may reduce serum cholesterol levels and the risk of coronary heart diseases and diabetes. Moreover, large segments of the population in developing countries suffer from protein malnutrition. Projections, based on current trends, indicate a widening gap between the human population and protein supply. Hence, intense research efforts are currently being directed toward identifying and evaluating underexploited sources, such as alternative protein crops for the world of tomorrow (Siddhuraju, Vijayakumari, & Janardhanan, 1996). In this regard, various studies have been carried out to assess the potential of legumes that are still not widely used as dietary sources of protein; moreover, these may constitute a genetic resource for the improvement of traditional legume

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crops (Agbede & Aletor, 2005; Guzman-Maldonado, Acosta-Gallegos, & Paredes-Lopez, 2000; Siddhuraju et al., 1996; Wong & Cheung, 1998).

The demand for plant-derived ingredients in food formulation has increased tremendously, thanks to greater knowledge of their functional properties and processing and nutritive value. Seeds of the genus *Lupinus* contain 30–40% protein (Hill, 1977; Martínez-Villaluenga, Frıas, & Vidal-Valverde, 2006; Porres, Aranda, López-Jurado, & Urbano, 2005; Van Barneveld, 1999), a level that is not far from that of soybean. Sweet lupin, such as *Lupinus albus* var. *multolupa,* is considered suitable for human consumption, since it has low levels of bitter-tasting and potentially toxic alkaloids (Petterson, 1998). In addition, lectins and protease inhibitors, which can reduce protein digestibility, are found at lower levels in lupin than in many other legumes (Martínez-Villaluenga et al., 2006; McPhee & Muehlbauer, 2002; Petterson, 1998). However, a potentially detrimental aspect of the use of lupins in products is their manganese content. *L. albus* specie has 20 times more manganese than do other lupin species (Hung, Handson, Amenta, Kyle, & Yu, 1987; Hung, Handson, Amenta, Kyle, & Yu, 1988). There have been reports in human and animal studies of the adverse effects of high manganese dietary intake, which can cause loss of appetite and reduced growth of sheep, pigs and poultry (Batterham, 1979;Grace, 1973;Karunajeewa & Barlett, 1985), as well as neurotoxicity in humans (McMillan, 1999; Stredrick et al., 2004). For these reasons, *L. albus* could be restricted to the production of lupin protein isolate, as the manganese, being in a soluble form, remains in the aqueous phase and is removed during precipitation of proteins (Hung et al., 1987; Hung et al., 1988).

Although lupin seeds have high levels of protein, rat studies have shown that the protein quality of sweet lupin is inferior to that of egg protein and casein (Fudiyansyah, Petterson, Bell, & Fairbrother, 1995; Yen, Grant, Fuller, & Pusztai, 1990). This low protein quality can be partially explained by the low proportions of essential sulfur containing amino acids, methionine and cystine, in the kernel protein of the lupinus species (Gueguen & Cerletti, 1994; Molvig et al., 1997; Trugo, Donangelo, Trugo, & Bach Knudsen, 2000). Nevertheless, a recent study in human subjects highlighted the high bioavailability of protein from a low-alkaloid *L. albus* variety (Mariotti, Pueyo, Tome, & Mahe, 2002).

The organoleptic properties of a diet are influenced by technological processes and have to be taken into consideration in order to ensure an adequate food intake in studies aimed at evaluating protein quality. Odour may be one of the limiting factors in rat food intake. The oxidation of fatty acids becomes crucial during food storage and affects the sensorial quality of a meal, as well as its nutritional quality, by the formation of hydroperoxides reacting with amino acid side-chains to give non-digestible products (Millan, Alaiz, Hernandez-Pinzon, Sanchez-Vioque, & Bautista, 1995).

The aim of the present study was to carry out the chemical and biological evaluation of lupin protein isolates (LPI) in growing rats, using diets containing LPI as the sole protein source in order to establish the nutritive quality of the isolate, and the potential beneficial effect of supplementation with 0.5% ascorbic acid, on daily food intake.

2. Materials and methods

2.1. Plant material

Lupin seeds (*L. albus* var. *multolupa*) were provided by the Agrarian Research and Technology Development Service from the Agriculture and Commerce Council of the Junta de Extremadura (Spain). Seeds were cleaned and stored in polyethylene containers at 4 °C until used.

2.2. Preparation of lupin protein isolates

Lupin protein isolates (LPI) were prepared by alkaline water extraction and isoelectric precipitation. Lupin seeds were milled and sieved through 60-mesh. One hundred grams of lupin (*L. albus* var. *multolupa*) flour was suspended in 1 l of distilled water containing 0.025% (p/v) Na_2SO_3, then adjusted to pH 9.0 using 1 M NaOH. The suspension was stirred for 1 h at room temperature, and then was centrifuged at 3000*g* for 30 min. In order to obtain higher yields, the extraction and centrifugation were repeated on the residue. The extracts were combined and acidified to pH 4.5 using 1 M HCl. The precipitate was recovered by centrifugation at 3000*g* for 30 min, then neutralized by 1.0 M NaOH to pH 7 and washed with distilled water, several times. The neutralized precipitate was freeze-dried and then milled using a household mill (Braun, Germany), sieved through 60-mesh and finally stored under vacuum (LPI A). In the lupin protein isolates treated with ascorbic acid (LPI B and LPI C), 0.5% ascorbic acid was added after freeze-drying and prior to the milling process. Regarding LPI C, lupin flour was defatted with hexane as a first step of lupin protein isolate preparation, and further hexane evaporation from lupin flour was carried out at room temperature.

2.3. Analytical methods

2.3.1. Chemical analyses

Moisture content of the different lupin protein isolates and diets was determined by drying to constant weight in an oven at 105 ± 1 °C. Fat content of protein isolates was determined according to the Soxhlet method (AOAC, 1990). Total nitrogen was determined according to Kjeldahl's method. Analytical results for nitrogen were validated by two different standard reference materials (haricot verts beans, RM 383, and synthetic feed for growing pigs, BCR 709. Community bureau of reference, commission of European communities) (mean ± SD value of five independent replicates of RM 383 was 1.03 ± 0.01 g/100 g DM versus

certified value ± uncertainty range for N of 1.05 ± 0.02 g/100 g DM; mean ± SD value of five independent replicates of BCR 709 was 19.7 ± 0.32 g/100 g DM versus certified value ± uncertainty range for crude protein of 19.9 ± 0.5 g/100 g DM). Ash content was measured by calcinations at 450 °C to a constant weight. Samples of ashed material were dissolved in 6 N HCl before analyses. Manganese content was determined by atomic absorption spectrophotometry, using a Perkin–Elmer Analyst 300 spectrophotometer. Analytical results for ash and Mn content were validated by the two previously described standard references, RM 383 and BCR 709; in the case of total ash (mean ± SD value of five independent replicates of RM 383 was 2.48 ± 0.01 g/100 g DM versus certified value ± uncertainty range of 2.4 ± 0.1 g/100 g DM; mean ± SD value of five independent replicates of BCR 709 was 4.29 ± 0.07 g/100 g DM versus certified value ± uncertainty range of 4.2 ± 0.4 g/100 g DM), and one standard reference of whole meal flour in the case of Mn (CRM-189, community bureau of reference, commission of European communities) (mean ± SD value of five independent replicates was 63.9 ± 1.30 mg/kg dry matter versus certified value ± uncertainty range of 63.3 ± 1.6 mg/kg). Amino acid content of protein isolated was analyzed by high-performance liquid chromatography (HPLC), as described by Rozan, Kuo, and Lambein (2000). The dry weights of the samples were approximately 50 mg. Two hundred microlitres of DL-norleucine (200 μmol/ml, Sigma) was added to samples as internal standard. Protein hydrolysis was done by following a classical method with 6 N HCl for 21 h at 110 °C in a vacuum closed vial. Hydrolysates were dried under vacuum and rinsed twice with water.

2.3.2. Biological methods

2.3.2.1. Diets. The different experimental diets were isocaloric and isonitrogenous, with the lupin protein isolates (LPI A, LPI B or LPI C) or a reference protein (casein) as the sole protein source. The isonitrogenous level was achieved by taking in account the composition of the protein source (Table 1). In all cases, and taking into consideration the results of the amino acid profile with a low chemical score (12.8–16.7%) in which Cys + Met were the limiting amino acids; the experimental diets were supplemented with 0.5% L-methionine. The other dietary ingredients were added, following the recommendations of the American Institute of Nutrition (Reeves, Nielsen, & Fahey, 1993). The formulation of experimental diets is collected in Table 3. The low amount of LPI in the diets, a consequence of their high protein levels, made it necessary to add a mineral–vitamin premix supplement in order to meet the nutrient requirements of growing rats. Experimental diets LPI B and LPI C were prepared and fed to the animals within two weeks of ascorbic acid addition to the protein isolates. During the storage and experimental periods, the protein isolates and experimental diets were kept refrigerated in darkness (see Table 2).

2.3.2.2. Experimental design. The experimental procedure was similar to the rat balance method described by McDonough et al. (1990), recording changes in body weight and food intake and then calculating nitrogen intake and fecal and urinary nitrogen excretion. Protein quality of the LPI A diet was assessed, taking a casein–methionine control diet with similar nitrogen content and fed *ad libitum* to the animals as a control (C + M 1). Due to the low daily food intake exhibited by the animals in the experimental group LPI A, a new experimental group fed with a casein–methionine control diet, *pair-fed* to the average daily food intake by the animals in the LPI A group, was included in the experimental design (C + M 2) (Table 3). With the aim of

Table 2
Amino acid composition (g/16 g N) of lupin seed flour and protein isolates from *Lupinus albus* var. *multolupa*

Amino acids (g/16 g N)	Lupin seed	LPI A	LPI B	LPI C
Non-essential				
Asp	7.12	10.1	10.3	11.7
Glu	18.4	25.6	26.0	29.7
Ser	4.38	5.07	5.16	5.98
Gly	3.04	3.15	3.20	3.69
Arg	3.27	10.7	10.9	11.1
Ala	3.49	3.05	3.10	3.94
Pro	3.01	3.64	3.71	4.32
Essential				
His	2.46	2.09	2.09	2.43
Ile	2.19	3.51	3.57	4.01
Leu	3.82	6.59	6.70	7.55
Lys	1.63	2.61	2.66	2.79
Thr	2.45	2.89	2.84	3.15
Trp	3.20	3.73	3.83	4.09
Val	3.27	2.96	3.01	3.48
Met + Cys	0.62	0.84	0.86	1.09
Tyr + Phe	3.66	6.06	6.17	6.76

Table 1
Chemical composition of raw seed flour and lupin protein isolates (LPI)[d]

	Raw seed flour	LPI A	LPI B	LPI C
Total-N (g/100 g DM)	5.58 ± 0.03[a]	14.3 ± 0.10[b]	14.0 ± 0.07[b]	15.7 ± 0.05[c]
Fat (g/100 g DM)	14.6 ± 0.35[c]	10.72 ± 0.42[b]	9.92 ± 0.42[b]	0.87 ± 0.04[a]
Ash (g/100 g DM)	3.51 ± 0.02[b]	3.65 ± 0.06[c]	3.65 ± 0.06[c]	1.69 ± 0.02[a]
Mn (mg/100 g DM)	90.1 ± 1.3[c]	24.5 ± 0.48[b]	24.5 ± 0.48[b]	9.39 ± 0.18[a]

[a,b,c] Means within the same line with different superscripts differ significantly ($P < 0.05$).
[d] Results are expressed in dry matter. Values are means ± SEM of five independent replicates.

testing whether ascorbic acid supplementation to the lupin protein isolates (LPI B; LPI C) could be beneficial for daily food intake by the experimental animals, three new experimental diets were designed: LPI B, LPI C and a casein–methionine control diet, with similar nitrogen content, supplemented with equal levels of ascorbic acid to those supplemented to the LPI B and LPI C diets (Table 3). All the experimental diets, with the exception of C + M 2, were feed *ad libitum*. The first three days of the experimental period were established as acclimatisaton, during which the rats were allowed to adapt to the diet and experimental conditions, followed by a 7-day balance period in which body weight and food intake was recorded and feces, urinary output and spilled food collected daily and separately for each rat and frozen at −70 °C. The frozen rat feces were freeze-dried, weighed and ground.

2.3.2.3. Animals. In each experimental group, 10 young albino Wistar rats (five males and five females) were used (total $n = 60$ for the six experimental groups tested). Recently weaned animals, with an initial body weight of 60 ± 1.5 g, were housed from day 0 of the experiment in individual stainless steel metabolic cages designed for separate collection of feces and urine. Cages were located in a room with a 12 h light/dark cycle, at a temperature of 21 ± 2 °C, fitted with an appropriate ventilation system. After completion of the feeding experiment, the rats were deprived of food for 16 h, weighed, anaesthetized with CO_2, and sacrificed. All experiments were undertaken according to Directional Guides Related to Animal Housing and Care (ECC, 1986).

2.3.2.4. Biological indices. The following indices and parameters were determined for each group according to the formulas given below: intake (expressed as dry weight), body weight gain, food transformation index (FTI, food intake in grams per day/weight gain in grams per day), apparent digestibility coefficient (ADC) (i) nitrogen retention (nitrogen balance) (ii) percent of nitrogen retention/nitrogen absorption (%R/A) and (iii) percent of nitrogen retention/nitrogen intake (%R/I) (iv)

$$\mathrm{ADC} = [(I - F)/(I)] \times 100, \quad \text{(i)}$$

$$\mathrm{Balance} = I - (F + U), \quad \text{(ii)}$$

$$\%\mathrm{R/A} = \{[I - (F + U)]/(I - F)\} \times 100, \quad \text{(iii)}$$

$$\%\mathrm{R/I} = \{[I - (F + U)]/(I)\} \times 100, \quad \text{(iv)}$$

where I = intake, F = fecal excretion, and U = urinary excretion.

Table 3
Ingredients and composition of casein control and lupin protein isolate (*Lupinus albus* var. *multolupa*) diets (g/100 g DM)

Ingredients	C + M 1; C + M 2	LPI A	C + M 3	LPI B	LPI C
Casein	13.2	–	13.2	–	–
Lupin protein isolate	–	13.2	–	13.8[b]	12.5[b]
Corn starch	59.8	61.5	59.8	60.9	60.3
Sucrose	10.0	10.0	10.0	10.0	10.0
Olive oil	7.0	5.3	7.0	5.6	7.0
Cellulose	5.0	5.0	5.0	5.0	5.0
Mineral mix[a]	3.5	3.5	3.5	3.5	3.5
Vitamin mix[a]	1.0	1.0	1.0	1.0	1.0
Choline bitartrate	0.25	0.25	0.25	0.25	0.25
L-Methionine	0.5	0.5	0.5	0.5	0.5
Ascorbic acid	–	–	0.065	–	–

[a] According to Reeves et al. (1993).
[b] An average of 0.065 g ascorbic acid per 100 g of diet was supplied by lupin protein isolates B and C.

2.4. Statistical analysis

Experimental data were analyzed by one-way ANOVA and Duncan's multiple range test, using statgrafic 5.0 system software (statistical graphics corporation, Rockville, MD). Differences were considered significant, when $P < 0.05$.

3. Results and discussion

3.1. Chemical analyses

The composition, as total nitrogen, fat, ash and Mn, of lupin isolates is described in Table 1. The lupin products obtained exhibited a high protein content (89–98%), whereas the fat and ash contents varied considerably among the different lupin protein isolates, with the lowest values being found for the ether-extracted lupin protein isolate (LPI C). The Mn content in protein isolates was dramatically decreased by protein isolation (73% in LPI A and LPI B), when compared to the raw seed flour content. A further Mn loss was achieved by the combination of organic solvent extraction of fat and aqueous extraction and isoelectric precipitation of proteins in LPI C (90%). Lupin protein isolates were rich in non-essential amino acids, e.g. aspartic acid, glutamic acid and arginine (11%, 28% and 11% of the total amino acid content, respectively). With regard to the essential amino acid content, methionine and cystine were the limiting amino acids in the lupin seed flour, as well as in the three protein isolates studied.

Chemical composition of the lupin flour used in the present experiment was within the range of values reported in the literature (Hill, 1977; Martınez-Villaluenga et al., 2006; Porres et al., 2005; Van Barneveld, 1999). Protein isolation counterbalanced the potential negative effect of an excessive Mn content of the lupin variety selected, due to Mn leaching to the aqueous extraction solvent, which is removed during precipitation of proteins (Hung et al., 1987; Hung et al., 1988). The lower essential amino acid content of *L. albus* var. *multolupa* protein isolate used in the present experiment, when compared to the results reported by Chew, Casy, and Johnson (2003) for protein isolates from *Lupinus angustifolius* var. *gungurru,* with the exception of tryptophan content, which was higher in the

protein isolates from *L. albus* var. *multolupa*, could be due to differences in the lupin species and varieties used for protein isolate preparation. Protein isolation led to a considerable increase in the contents of all amino acids, when compared to the raw lupin flour, an increase that should lead to improvement in the nutritive quality of the protein.

The Leu/Ile and Leu/Lys ratios are useful indices that may help to ascertain the nutritional quality of proteins. An excess of leucine impairs the adequate utilization of isoleucine and lysine (Harper, Benton, & Elvbejhem, 1955; Petterson, Sipsas, & McIntosh, 1997). The Leu/Ile and Leu/Lys ratios were 1.88 and 2.6, respectively, in the protein isolates studied. These values are similar to or only slightly higher than the results reported for other legumes in the literature, such as faba bean, chickpea, bean or lentil (Fernandez, Lopez-Jurado, Aranda, & Urbano, 1996; Nestares, Barrionuevo, Urbano, & López-Frıas, 2001; Nestares, Lopez-Frias, Barrionuevo, & Urbano, 1996; Porres et al., 2002), and indicated the potential good protein quality of lupin.

Nevertheless, despite the favourable Leu/Ile and Leu/Lys ratios and the higher chemical score of lupin protein isolates, when compared to the raw lupin flour, the protein qualities of the isolates, from a nutritional point of view, were not high due to their limiting levels of sulfur-containing amino acids Met + Cys, when compared to the nutrient requirements of the growing rat (6.53 g/16 g N) (NRC, 1995), the 1-year-old infant (4.2 g/16 g N) (FAO/WHO, 1991), or the human adult (1.7 g/16 g N) (FAO/WHO, 1991), as well as their low levels of other essential amino acids, e.g. valine or lysine.

3.2. Biological assays

3.2.1. Food intake

Daily food intake of rats fed, *ad libitum,* the LPI A diet was significantly less than that obtained from a casein–methionine control diet with similar protein content and fed *ad libitum* (C + M 1) (Table 4). This lower dietary intake led to impaired growth, as shown by the low values of daily weight gain and food transformation index (FTI) of the former experimental group. Compared to the casein–methionine control group with a similar daily food intake (*pair fed*) (C + M 2), the growth and nutritive utilization of protein by the animals fed the LPI A diet were significantly lower, despite high and similar levels of digestive utilization (ADC) of protein by the two experimental groups (C + M 2, LPI A) that resulted in similar levels of net nitrogen absorption. Food intake and weight gain increased very significantly with the addition of 0.5% ascorbic acid to isolate B compared with lupin protein isolate A. Likewise, fat extraction, prior to the protein isolation process, and further addition of ascorbic acid (0.5%) to the protein isolate (LPI C), led to an increment in food intake that was similar to the daily food intake attained by the experimental group fed the LPI B diet and the experimental groups that consumed a casein–methionine diet, with or without ascorbic acid supplementation fed *ad libitum* (C + M 1; C + M 3).

The reduced food intake by the experimental group that consumed the LPI A diet could be due to poor palatability caused by fatty acid oxidation of the lupin protein isolate during the storage and experimental periods, which led to a decrease in its nutritional quality, as confirmed by the fact that supplementation of lupin protein isolate with 0.5% of an antioxidant, such as ascorbic acid, and storage of the LPI under vacuum and refrigeration in darkness in order to reduce fatty acid oxidation during the storage period, led to a significant increase in daily food intake by the animals fed the LPI B and LPI C diets that matched the daily food intake attained by the animals fed the casein–methionine control diets (C + M 1; C + M 3). Supplementation of 0.5% ascorbic acid could be, in part, responsible for the improved daily food intakes obtained in the LPI B and LPI C experimental groups. However, that did not appear to be the case under the experimental conditions of the present study, given that supplementation to the C + M 3 diet with similar levels of ascorbic acid to those

Table 4
Food intake, weight gain and nutritive utilization of protein by rats fed casein or lupin protein isolate (*Lupinus albus* var. *multolupa*) diets

	C + M 1	LPI A	C + M 2	C + M 3	LPI B	LPI C
Total-N (g/100 g)	1.99 ± 0.02^{a}	1.92 ± 0.03^{a}	1.92 ± 0.02^{a}	2.01 ± 0.03^{a}	1.95 ± 0.01^{a}	1.90 ± 0.04^{a}
Intake (g/d)	13.21 ± 0.53^{b}	7.15 ± 0.21^{a}	7.34 ± 0.01^{a}	12.72 ± 1.27^{b}	13.07 ± 0.36^{b}	12.15 ± 0.32^{b}
Weight gain (g/d)	4.85 ± 0.36^{d}	0.57 ± 0.03^{a}	2.52 ± 0.10^{b}	4.57 ± 0.26^{d}	4.44 ± 0.23^{d}	3.37 ± 0.17^{c}
FTI	2.78 ± 0.10^{a}	12.54 ± 0.20^{c}	2.91 ± 0.12^{a}	2.85 ± 0.11^{a}	2.99 ± 0.10^{a}	3.67 ± 0.14^{b}
N intake (mg/d)	263 ± 10.52^{c}	142 ± 4.22^{a}	146 ± 0.11^{a}	256 ± 8.06^{c}	255 ± 6.96^{c}	230 ± 5.99^{b}
Fecal N (mg/d)	13.2 ± 0.70^{c}	9.66 ± 0.75^{a}	10.8 ± 0.51^{ab}	12.6 ± 0.84^{bc}	17.1 ± 1.06^{c}	14.1 ± 0.58^{c}
Urinary N (mg/d)	58.9 ± 3.43^{bc}	75.8 ± 3.30^{c}	37.7 ± 2.07^{a}	57.5 ± 4.83^{b}	77.2 ± 7.91^{c}	63.8 ± 10.45^{bc}
Absorbed N (mg/d)	250 ± 10.05^{c}	132 ± 3.78^{a}	135 ± 0.57^{a}	243 ± 7.53^{c}	238 ± 6.27^{c}	216 ± 5.68^{b}
ADC (%)	95.0 ± 0.20^{c}	93.2 ± 0.43^{ab}	92.6 ± 0.35^{a}	95.1 ± 0.24^{c}	93.3 ± 0.31^{ab}	93.9 ± 0.21^{b}
Retained N (mg/d)	191 ± 7.64^{d}	56.2 ± 3.68^{a}	97.7 ± 3.12^{b}	186 ± 3.94^{d}	160 ± 6.44^{c}	153 ± 9.40^{c}
% R/A	76.5 ± 0.88^{c}	42.4 ± 2.27^{a}	72.2 ± 1.56^{bc}	76.6 ± 1.33^{c}	67.8 ± 2.98^{b}	70.8 ± 4.37^{bc}
% R/I	72.6 ± 0.84^{c}	39.7 ± 3.36^{a}	66.9 ± 2.31^{bc}	72.8 ± 1.30^{c}	63.3 ± 2.77^{b}	66.5 ± 4.13^{bc}

Results are means ± SEM ($n = 5$ for diet content and $n = 10$ for biological parameters and indices). Means within the same line in each individual experiment with different superscripts differ significantly ($P < 0.05$). FTI, food transformation index, ADC, apparent digestibility coefficient, %R/A, percentage of retention to absorption, %R/I, percentage of retention to ingestion.

present in LPI B and LPI C diets did not improve daily food intake, when compared to the C + M 1 diet, to which no supplemental ascorbic acid was added.

Therefore, the results obtained in this study suggest that supplementation of lupin protein isolates with small quantities of ascorbic acid, that were within the range of commonly accepted concentrations regarded as safe, was an efficient and feasible way of preserving their organoleptic properties during the 2–4 weeks of the storage-experimental period, thus maintaining an appropriate daily food intake. The effect of ascorbic acid could occur by the restriction of free radicals at the beginning of storage (Lamghari et al., 1997), and would delay lipid oxidation without preventing it indefinitely.

3.2.2. Digestive utilization of protein

Digestive utilization of nitrogen from protein isolates B and C was high, similar to that obtained for the LPI A diet, and slightly lower than that obtained for the experimental groups fed the casein–methionine control diet *ad libitum* (C + M 1; C + M 3).

In contrast to reports that describe legumes as having a low protein digestibility compared to animal protein (Mongeau, Sarwar, Peace, & Brassard, 1989), the lupin protein isolates B and C in the present study exhibited acceptable levels of digestibility that were close to those obtained with a casein–methionine control diet (C + M 1; C + M 2; C + M 3) and higher than those reported for a range of other legumes (Friedman, 1996). Dietary trypsin inhibitors in legumes are often responsible for the poor digestibility of dietary protein by interference with the proper function of trypsin, leading to growth inhibition and pancreatic hypertrophy (Liener, 1994). Chew et al. (2003) reported that the high digestibility of lupin protein concentrates may be explained by the fact that antinutritional factors, such as lectins and protease inhibitors, are found at relatively low levels in lupin compared to many other legumes.

3.2.3. Metabolic utilization of protein

The urinary excretion of nitrogen was significantly greater for the group of rats fed the LPI A diet, when compared to the C + M 1 experimental group, which, at a metabolic level, led to significantly lower values of the indices used to evaluate protein quality (nitrogen retention, %R/A, %R/I) under our experimental conditions. The urinary excretion of nitrogen in the experimental groups fed the LPI B and LPI C diets was slightly higher than those of the C + M 1 and C + M 3 experimental groups, which, together with the lower net absorption of this nutrient by the LPI B and LPI C groups compared to the casein–methionine controls, resulted in a numerically lower nitrogen balance and %R/A. Nevertheless, the metabolic utilization of nitrogen by the experimental groups fed the LPI B and LPI C diets was substantially greater than that obtained for the experimental group fed the LPI A diet.

Supplementation of ascorbic acid to the casein–methionine control diet (C + M 3) did not induce any effect in any of the biological indices used to study the nutritional quality of protein, when compared to the unsupplemented C + M 1 diet.

Under our experimental conditions, the low metabolic utilization of protein attained by the experimental group fed the LPI A diet, compared to the casein–methionine *pair-fed* (C + M 2) and *ad libitum* (C + M 1) control groups, can be attributed to a combination of low daily food intake and the amino acid imbalance of the lupin protein with respect to the nutrient requirements of the growing rat (NRC, 1995), an imbalance that is especially evident for Lys, Thr and Val. The potential use of dietary protein for energetic rather than plastic needs cannot be ruled out, as shown by the higher percentage of retained to absorbed or ingested nitrogen (%R/A; %R/I) of the animals that consumed the C + M 2 diet, when compared to those fed the LPI A diet. Nevertheless, under our experimental conditions, it appeared that the amino acid imbalance factor was mainly responsible for the low nutritive utilization of protein achieved by the animals fed the LPI A diet.

The protein quality of the LPI B and LPI C diets, judged by the %R/I index, was slightly lower than for the C + M control diets fed *ad libitum*, but markedly superior to that of the LPI A diet. This finding corroborates the idea that an essential amino acid imbalance of a specific protein can be partially compensated by an improved daily food intake, as previously seen in the %R/A index. Similar observations were previously reported by Bender (1956), who obtained a high correlation between daily food intake and nutritional quality of the protein source, as assessed by the protein efficiency ratio or net protein utilization indices.

In spite of a similar daily food intake and nutritive utilization of protein, daily weight gain and the nutritional index of FTI obtained for the experimental group fed the LPI C diet were inferior ($P < 0.05$) to the values obtained for the group fed the LPI B or the casein–methionine control groups fed *ad libitum* (C + M 1; C + M 3), among which no significant differences were found. Nitrogen retention by the animals fed the LPI B and LPI C diets was not correlated with weight gain or FTI, given that significant differences in the above- mentioned nutritional indices were found, in spite of similar nitrogen balances. The only differences between the LPI B and LPI C diets were in the amounts of exogenous fat added (5.7 versus 7 g/100 g DM in LPI B and C, respectively), and the fat extraction process applied to lupin flour prior to protein isolation in the LPI C.

4. Conclusions

The potential harmful effects of the high Mn content present in the sweet lupin seeds used for the present study (*L. albus*, var. *multolupa*) were significantly averted by lupin protein isolation that led to a considerable reduction in Mn content and considerable increments in the levels of

essential and non-essential amino acids. Supplementation of 0.5% ascorbic acid to the lupin protein isolates resulted in a significant improvement in their palatability. This was reflected in a significant increase in daily food intake, which, together with the good quality of its protein shown by the biological indices assayed, as well as its low levels of Mn, make lupin protein isolates an excellent protein source for human and animal nutrition.

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