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Chemical Composition, Nutritive Value, and Toxicological Evaluation of Two Species of Sweet Lupine (*Lupinus albus* and *Lupinus luteus*)

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Two species of sweet Lupine, *Lupinus albus* and *Lupinus luteus*, were analyzed. Both species were good sources of protein (34 and 39%). Lipid content measured as ether extract was 10.9% for *L. albus* and 4.7% for *L. luteus*. Both legumes had high crude fiber contents of more than 10% and low alkaloid contents (0.05 and 0.09%). The protein efficiency ratio was low in both species (0.48 and 0.99), but supplementation with DL-methionine increased base values significantly ($p < 0.01$), 2.84 and 2.30, respectively. In a toxicity study with rats that consumed a 20% lupine protein diet (supplemented with 0.3% DL-methionine), the growth rate of animals fed *L. luteus* and *L. albus* was similar to that produced by an unsupplemented 20% protein casein diet. The weight of liver, kidneys, heart, spleen, and adrenals and the histology of kidneys and lungs were normal.

For centuries legumes have been an important source of protein and calories for many peoples of the world. Lupines among the legumes were used as a human food by ancient cultures surrounding the Mediterranean and by those people living in the Andes highlands (Grindley and Akour, 1955; Castillo, 1965), and among the vegetable crops legume grains contain the highest percentage of protein. However, the proteins of legumes are generally considered good sources of lysine, and generally low in the sulfur-containing amino acids. Some lupines referred to here as "bitter lupines" also contain high levels of the alkaloids lupanine and spartein which impart a bitter taste. Sweet lupines, or the low alkaloid species, have been developed by genetic selection (Gladstones, 1972; von Baer, 1972) and because their composition compares favorably with soybeans, sweet lupines could become an important source of protein and oil for human diets since their taste is acceptable. Their high seed yield and good growing and harvesting characteristics also make them suitable for cultivation in many areas of the world.

Different native and genetically selected lupine cultivars have been used as sources of protein in animal feeding trials with rabbits, pigs, and broilers (Flores, 1970; Pearson and Carr, 1977; Yule and McBride, 1976). The animals' productive responses were, for the most part, adequate.

In Chile, efforts are currently underway to introduce lupine flour into substitute milk formulas for infants and into various common foods. This prompted us to evaluate the chemical, nutritional, and toxicological properties of two species of sweet lupines grown in Chile: *Lupinus albus* and *Lupinus luteus*. Presented here are the results of this investigation.

MATERIALS AND METHODS

Samples of *L. luteus* and *L. albus* obtained from a local grower were ground with a laboratory hammer mill (Wiley Laboratory Standard Model 4) and passed through a 100-mesh sieve. Moisture, ether extract, total ash, and crude fiber were determined according to AOAC (1970) methods. Nitrogen was determined by a macroKjeldahl procedure, followed by a semimicrodistillation into a 2% boric acid solution with a mixed indicator (Markham,

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Table I. Composition of Experimental Diets at 10% Protein Level (g/kg)

	L. albus		*L. luteus*		casein, E
	A	B	C	D	
casein[a]					115
L. albus	290	290			
L. luteus			256	256	
DL-methionine[b]		3		3	
nonnutritive fiber[a] (Alphacel)	50	50	50	50	50
corn oil[c]	68	68	88	88	100
vitamin mix (Chapman et al., 1959)	10	10	10	10	10
mineral mix[d]	40	40	40	40	40
corn starch	542	539	556	553	685

[a] Nutritional Biochemicals Corp., Cleveland, OH. [b] Sigma M-9500; minimum purity, 99.5%. [c] Mazola Corn Products, Chile. [d] Mineral mix: USP XIV. General Biochemicals, Chagrin Falls, OH.

Table II. Composition of Diets Used in the Toxicity Test at a 20% Protein Level[a]

	diet I, casein	diet II, *L. albus*	diet III, *L. luteus*
casein	230		
L. albus		581	
L. luteus			513
DL-methionine		3	3
nonnutritive fiber (Alphacel)	50		
corn oil	100	37	76
vitamin mix	10	10	10
mineral mix	40	40	40
corn starch	570	329	358

[a] See Table I for nutrient sources.

1942). The factor 6.25 was used to convert nitrogen to protein. Alkaloids were extracted at different pH values (Svoboda et al., 1959) and titrated by acid–base potentiometry (Reilley and Sawyer, 1961).

Amino Acid Analysis. Samples for amino acid analysis were hydrolyzed with 6 N HCl at 110 °C for 22 h (Kohler and Palter, 1967), and the hydrolyzate was analyzed with an Hitachi Perkin-Elmer amino acid analyzer (Model KLA-3B) based on the principle of Spackman et al. (1958).

The biological quality of the protein of both cultivars using the protein efficiency ratio (PER) was determined according to the procedure of Chapman et al. (1959). Rats of the Charles River strain (21–23 days old) were used in this study. Ten animals per group were randomly assigned to the different treatments and were individually housed in screen-bottomed cages in an air conditioned room maintained at 23–25 °C. The composition of the experimental diets, shown in Table I, was designed to provide 10% protein. A reference ANRC casein diet was used as standard. All diets were fed ad libitum during the 28-day experimental period. Weekly records of individual food consumption and weight gain were kept.

Amino Acid Supplementation. The effect of methionine supplementation at the levels of 0.1, 0.2, and 0.3% on the protein efficiency ratio of *L. albus* protein was also measured. *L. Luteus* was supplemented at the level of 0.3% DL-methionine.

Protein digestibility was determined in all experimental diets supplemented with 0.3% DL-methionine and in the casein diet. The feces of each rat were collected weekly, pooled, and analyzed for nitrogen content. Digestibility was calculated according to the formula $D = (I - F/I)100$, where I and F represent dietary nitrogen intake and fecal nitrogen, respectively.

Table III. Percentage Chemical Composition of Two Species of Lupine: *L. albus* and *L. luteus*

	L. albus, g/100 g	*L. luteus*, g/100 g
water	13.0	12.5
ash	3.2	3.5
protein (N × 6.25)	34.4	39.0
ether extract	10.9	4.7
crude fiber	11.7	16.8
N-free extract[a]	26.8	23.5

[a] For difference.

Table IV. Alkaloid Content[a] of Two Species of Sweet Lupines (g/100 g)

chloroform extract, pH	*L. albus*	*L. luteus*
acid 2–3	0.013	0.029
neutral 7–8	0.038	0.059
basic 12		0.003
total	0.051	0.091

[a] As lupanine.

Table V. Amino Acid Composition of Two Sweet Lupines (*L. albus* and *L. luteus*)[a]

amino acid	*L. albus*	*L. luteus*
isoleucine	3.97	6.22
leucine	6.90	10.08
lysine	4.26	3.80
methionine	0.70	0.57
cystine	2.52	2.88
phenylalanine	3.65	4.40
tyrosine	4.40	2.32
threonine	3.32	3.52
valine	3.70	3.78
alanine	2.83	4.30
arginine	10.30	18.40
aspartic acid	9.45	12.80
glutamine	30.80	24.58
glycine	3.26	4.58
histidine	1.88	8.80
proline	4.62	4.77
serine	3.78	6.54

[a] Amino acid concentration is expressed in grams of amino acid/16 g of nitrogen.

The toxicity of *L. albus* and *L. luteus* at the level of 20% dietary protein supplemented with 0.3% DL-methionine was tested in a 112-day feeding experiment with rats using a 20% protein unsupplemented casein diet as a standard. Three groups of 12 animals each were fed one of the three protein sources: casein (diet I), *L. albus* (diet II), and *L. luteus* (diet III). The overall composition of the diets is shown in Table II. As in previous experiments, rats were housed individually, food and water were offered ad libitum, and diet intake and body weight were recorded weekly.

At the end of the experimental period, the animals were sacrificed and the weights of the livers, kidneys, spleens, hearts, and adrenals were recorded. Tissue samples of liver, kidneys, and lungs were fixed in 10% buffered formalin, processed through paraffin sectioned at 5 μm, and strained with hematoxylin eosine for microscopic examination.

Statistical Analysis. PER data were examined by the analysis of variance (Snedecor and Cochran, 1967) and Duncan's multiple range test (1955).

RESULTS

Both species of lupine had substantial amounts of protein and fat levels. *L. albus* contained less protein, more ether extractable material, and less crude fiber than *L.*

Table VI. Protein Efficiency Ratio (PER) and Protein Digestibility (D) of *L. albus* and *L. luteus*: Effect of Supplementation with Graded Levels of DL-Methionine[a]

	DL-Met, %	wt increase, g	PER	D
L. albus		6.0 ± 0.9[a]	0.48 ± 0.05[a]	
L. albus	0.1	70.4 ± 2.5[b]	2.86 ± 0.10[b]	
L. albus	0.2	65.0 ± 2.6[b]	2.89 ± 0.06[b]	
L. albus	0.3	66.5 ± 2.8[b]	2.84 ± 0.05[b]	74.9 ± 0.8
L. luteus		16.5 ± 1.2[c]	0.99 ± 0.08[c]	
L. luteus	0.3	59.5 ± 2.7[d]	2.30 ± 0.16[d]	74.1 ± 2.7
casein		56.5 ± 2.7[d]	2.76 ± 0.06[b]	87.0 ± 1.0

[a] Mean ± standard error. Means with the same letter in a column are not significantly different at the 0.05 level of probability based on Duncan's multiple range test.

Table VII. Performance of Rats Fed *L. albus*, *L. luteus*, or Casein at 20% Dietary Protein

	body wt gain week 1-6, g rat^{-1} day^{-1}	feed intake, week 1-6, g rat^{-1} day^{-1}	feed effic, week 1-6, wt gain/g of feed
L. albus + 0.3% DL-Met	2.75 ± 0.12	12.6 ± 0.56	0.26 ± 0.01
L. luteus + 0.3% DL-Met	3.09 ± 0.17	13.9 ± 0.54	0.26 ± 0.01
casein	2.82 ± 0.13	12.8 ± 0.54	0.28 ± 0.01

luteus (Table III). The total amount of alkaloids was very low in both species (Table IV), with *L. albus* containing about half the amount found in *L. luteus*.

The amino acid analyses (Table V) showed distinct differences between both species of lupines: *L. luteus* was higher in isoleucine, leucine, arginine, histidine, alanine, aspartic acid, glycine, and serine than *L. albus*, and lysine was higher in *L. albus* than *L. luteus*. Methionine was markedly low in both cultivars but cystine was approximately four times higher than methionine.

Protein Efficiency Ratio. Rats fed either lupine at the level of 10% dietary protein without methionine supplementation performed poorly (Table VI). The mean gain in body weight of rats on the diet containing *L. albus* was 6 g and for those fed *L. luteus* 16.5 g ($p < 0.05$). The values for PER were 0.48 and 0.99, respectively, as compared to 2.76 for casein ($p < 0.01$). Supplementation of *L. albus* with graded levels of DL-methionine caused a marked increase in both growth rate and PER. Supplementation with 0.1% DL-methionine resulted in growth and PER levels comparable to casein; however, supplementation levels of 0.2 and 0.3% DL-methionine did not produce further increases in these parameters. The performance of the animals fed *L. luteus* supplemented with 0.3% DL-methionine was, however, lower than those fed supplemented *L. albus*. The protein digestibility of *L. albus* and *L. luteus* supplemented with 0.3% DL-methionine was essentially the same for both species (Table VI).

Toxicity Test. The rate of growth of the animals fed the lupine diets at a level of 20% protein supplemented with 0.3% DL-methionine is shown in Figure 1. While the animals fed supplemented *L. luteus* gained weight practically at the same rate as those fed casein, those fed supplemented *L. albus* gained at a slightly lower rate (Table VII). Feed intakes and feed efficiencies of both lupines measured during weeks 1–6 were not different.

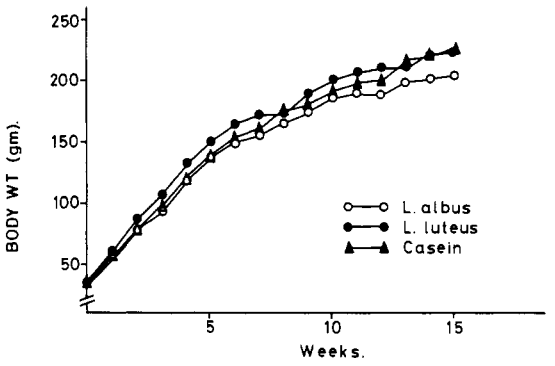

Figure 1. Growth of rats fed *L. albus*, *L. luteus*, or casein at 20% protein supplemented with 0.3% DL-methionine.

Organ Weights. No differences were observed in the organ-to-body weight ratios of liver, spleen, heart, and adrenal of rats fed either of the lupines or casein (Table VIII). After 112 days of feeding the experimental diets, gross autopsy findings, as well as microscopic examinations, were negative as to significant differences.

DISCUSSION

The protein content of both lupines tested closely resembles soybean. Lupine generally contains about twice the protein found in those legumes normally consumed by man. Additionally, lupine yields 1000–2000 kg/ha compared to 580–620 kg/ha for beans, or 760–870 kg/ha for chickpeas (Jalil, 1972). These facts may explain why lupines have been used for centuries as a human food by certain cultures. As with many legumes, however, lupines contain undesirable compounds that must be removed before consumption. The species used in the present study were sweet lupines, or low alkaloid, as shown by results given in Table IV. While bitter species contain about 3% alkaloids (Galdames, 1973), the cultivars examined in this study contained 0.05 and 0.09% total alkaloids. Our values are within the range of those found by other investigators in sweet lupines (Ruiz et al., 1977) and are significantly lower in comparison to bitter lupine (Galdames, 1973).

The nutritive value of legumes has been studied extensively (Bressani et al., 1973) and has been found to be variable and generally low in those studied. The main factor contributing to this variability appears to be the relatively low concentration of the sulfur amino acids in legume grains. The beneficial effect of the addition of methionine to legumes has been observed repeatedly (Jaffé, 1950a). As with most legumes, lupines have shown to be deficient in sulfur amino acids. Tannous and Cowan (1966) reported a value of 134 mg/g of N of total sulfur amino acids for lupines. In the current study, the amino acid composition of two samples of lupines (Table V) was within the normal range for these species (Hill, 1977). By comparison *L. luteus* contained higher levels of most amino

Table VIII. Organ Weight from Rats Fed DL-Methionine-Supplemented Lupine or Casein (Control) at 20% Dietary Protein at 112 Days of Experiment (g/100 g)[a]

diet	liver[b]	kidneys[b]	heart[b]	spleen[b]	adrenals[c]
casein	2.94 ± 0.12	0.63 ± 0.13	0.28 ± 0.02	0.20 ± 0.01	24.5 ± 3.3
L. albus	2.88 ± 0.09	0.66 ± 0.03	0.31 ± 0.05	0.19 ± 0.01	21.7 ± 2.6
L. luteus	2.98 ± 0.11	0.66 ± 0.02	0.28 ± 0.02	0.18 ± 0.02	19.5 ± 5.0

[a] All values were calculated as confidence limits of the mean according to the formula: $P[(\bar{x} + S_{\bar{x}}(t/2)] = 0.95$. [b] Grams/100 g of body weight. [c] Milligrams/100 g of body weight.

acids. Methionine was low in both lupines, especially *L. luteus*. This is in agreement with values reported elsewhere (Hill, 1977). Cystine, on the contrary, was present in a higher amount than that reported by other investigators (Aguilera and Trier, 1978). Thus sulfur amino acids would not appear to be limiting. However, PER values of both lupines without methionine supplementation were definitely low. When *L. albus* was supplemented with graded levels of DL-methionine, both growth rate and PER increased significantly. A similar result has been previously described (Hughes and Orange, 1976; Ruiz et al., 1977; Sgarbieri and Galeazzi, 1978). An explanation to this result could be the presence of amino acid structures involving cystine which are enzyme resistant, thus affecting the biological availability of this amino acid, as has been reported for other legumes (Kakade, 1974; Evans and Bandemer, 1967). It is important to point out that the addition of 0.1% DL-methionine produced a maximum response in both growth and PER and the levels of 0.2 and 0.3 DL-methionine did not further increase the biological quality. Although supplementation of *L. luteus* with 0.3% DL-methionine did produce a significant increase in growth rate and PER, the values remained significantly lower ($p < 0.05$) than those attained for *L. albus*. These lower values may be explained by the fact that lysine becomes limiting when adding high levels of methionine. This probably took place in *L. luteus*, which contains less lysine, more than in *L. albus*. On this basis the 0.1% level would have been more desirable. The results obtained in the long-term feeding test confirm in part this hypothesis (Table VI). Feed efficiency was similar for both lupines when the supplementation was 0.15% relative to protein. Protein digestibility measured for both lupines supplemented with 0.3% DL-methionine was practically identical although significantly lower ($p < 0.01$) than casein. Jaffé (1950b) reports a wide range of variation in the digestibility of different species and varieties of legumes, from as low as 59.5 for *Cajanus cajan* to as high as 93.9 for *Pisum sativum*.

The 112-day feeding study showed that the growth rate of the animals fed *L. luteus* supplemented with 0.3% DL-methionine was identical with that of rats fed casein, with that of the animals fed *L. albus* only slightly lower. Diet intake and feed efficiency were identical for the three groups of animals. These results indicate that sweet lupines fed to rats at the level of 20% dietary protein supplemented with 0.15% DL-methionine relative to protein have the same nutritive value as unsupplemented casein. In addition, the weight of liver, kidneys, heart, spleen, and adrenals was normal compared with control animals. The histological study of liver, kidneys, and lungs did not show cell alterations.

In summary, the protein of sweet lupines is of low quality but when supplemented with DL-methionine its biological quality is comparable to casein. In rats, the levels of alkaloids present in sweet lupines for 16 weeks did not adversely affect growth or histological and gross appearance of organs. Therefore, methionine-supplemented sweet lupines may be qualified to play a role in human nutrition as a source of both protein and calories.

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