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The use of Lupinus termis L. cultivated in Egypt, as a food protein supplement

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With 1 figure and 5 tables

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As a result of the rapid increase in the population, the diet of Egyptians suffers from unsufficient protein sources, particularly those of animal origin (1). The only solution is to augment the diet with legumes which, as a class, are relatively rich in protein as compared to most other classes of stuffs of vegetable origin.

A considerable amount of work has been carried out on evaluating the nutritive value of some leguminous seeds, and about their potential success as protein supplement in human nutrition.

Lupin (Lupinus termis) seeds are commonly consumed in the Middle East, their consumption is confirmed largely to the poorer class (2). The high protein content of the seeds makes them a rich source of dietary protein. The average annual crop of lupin for the year 1973 was 4500 tons.

For human consumption, the raw bitter seeds are boiled in water for half an hour, then steeped in running water for three days to remove the bitter taste of the seeds. Debittered seeds are slightly salted with common salt.

Experimental

I. Sampling and analysis

The raw lupins used through all these studies were brought from the local market. Each of the following treatments was carried out on two kilograms seeds of:

- 1) Raw lupin seeds (R.L.).
- 2) Raw decorticated seeds (R.D.L.).
- 3) Boiled decorticated seeds (B.D.L.), in which the seeds were boiled for one hour before decortication.
- 4) Debittered decorticated seeds (D.D.L.).

Immediately after respective treatments, all samples were dried in a vacuum oven at 60° C, moisture, nitrogen, fat, crude fiber, ash and iron content of seeds were determined according to the method described by A.O.A.C. (4). Calcium and phosphorus were determined as described by Stuffins (5).

The amino acids content of samples were assayed using the paper chromatography technique of Levy and Chung (6), after being hydrolysed with hydrochloric acid according to Khan and Baker (7). Cystine and Methionine were determined according to the method of Jamalian and Pellet (8). The colorimetric method of Blauthi, Chaezinski and Berbec (9) was followed for the estimation of Tryptophan.

Diet No.	A. A.	Suppl.	Raw		Raw decor	ticated	Boile	d	Debit	tered
	DL. meth.	L-lys.	Quantity added %	Protein % added						
2	=		24.7	10.0		-	_	_	_	_
3	_	-		_	22.1	10.0	-		_	_
4	_	_	-	_			20.6	10.0		-
5	_		_		_	-	_		21.6	10.0
6	0.5	0.25	22.8	9.25	_		_	_	_	_
7	0.5	0.25	_	_	21.0	9.25		_	_	_
8	0.5	0.25	_	~			19.1	9.25	_	_
9	0.5	0.25	_	-	_		_	_	20.1	9.25

Table 1. Supplement to the basal diet

II. Determination of toxic factors

a) Trypsin inhibitors

Seeds were extracted with ammonium formate-formic acid buffer at pH 3.2, the Trypsin inhibitors were assayed according to the method described by Laskowski (10).

- b) Hemagglutinating activity of seed extract was determined according to the method of *Leiner* and *Hill* (11), using rabbit red blood corpuscles.
- c) The presence of alkaloids was tested using Wagner's reagent. Their percentage was determined by the method of Jenkens, Christian and Hanger (12).
- d) Cyanogenetic glucosides were tested according to the method of Burnside (13).
- e) Saponines were determined as described by *Ain-shoka* (14). Alcoholic seed extracts were evaporated under vacuum and the residue was dissolved in normal saline. About 1 ml of solution was added to 10 ml of suspension of red blood corpuscles in normal saline (1 in 40). Hemolysis indicates the presence of saponins in the seeds.

III. Rat feeding experiments

For the determination of net protein utilization, the method given by *Miller* and *Bender* (15), was followed using hooded rats which were caged individually. One group of rats was fed the basal protein-free diet as shown in table 1, in which the protein sources under test were incorporated into the basal protein-free diet at the expense of maize starch at the level to contribute $10\,\%$ crude protein to the diet. After ten days the rats were killed and the net protein utilization was calculated after the analysis of carcasses nitrogen.

Results and discussion

Proximate analysis

Table 2 shows the nutrient content of raw lupin seeds. The crude protein content is 40.36%, which is high as compared with other leguminous seeds. Similar values were given by *Tannous* et al. (16). The fiber content of tested lupin was found to be 10.8%, which is fairly high. *Gladstone* (17) showed that the removal of the seed coat greatly reduces the fiber content in lupin.

Table 2. Proximate analysis of lupin seed calculated on dry weight basis*)

Item	g/100 g	
Protein (N x 6.25)	40.36	
Fat	9.71	
Carbohydrate by difference	27.13	
Fiber	10.78	
$\mathbf{A}\mathbf{s}\mathbf{h}$	3.39	
	mg/100 g	
Calcium	290	
Phosphorus	356	
Iron	6.5	

^{*)} Moisture content is 8.63%

Acid hydrolysis of raw and treated lupin seeds revealed the presence of the following amino acids, Alanine, Arginine, Aspartic acid, Cystine, Methionine, Glutamic acid, Glycine, Histidine, Tyrosine, Leucine + Isoleucine, Lysine, Phenylalanine, Serine, Threonine, Tryptophan and Valine as shown in table 3. Glutamic acid, Glycine and Serine are present in amounts similar to those previously reported by Jamalian and Pellet (8), and by Tannous et al. (16), while aspartic acid is present in higher amounts.

Comparing the amino acid content of whole raw seeds with that of the raw decorticated lupin indicated that the amino acids are nearly concentrated in the dicotyledon of the seed. Boiling the seeds resulted in small loss for most of the amino acids studied with the exception of Lysine and Tryptophan, which exhibited slight increase after boiling.

Table 3. Amino acids content of raw and treated lupin as compared with the provisional amino acid pattern recommended by the FAO (1955)*)

Item	Raw	Decorti- cated	Boiled	Debittered	FAO
Alanine	175	188	183	176	
Arginine	762	799	778	770	-
Aspartic acid	959	975	970	942	
Cystine	45	49	46	43	126
Methionine	112	125	114	112	144
Glutamic acid	1209	1229	1217	1194	_
Glycine	291	311	289	281	
Histidine	90	102	95	94	
Tyrosine	290	297	287	284	180
Leucine + Isoleucine	380	385	380	374	576
Lysine	215	225	228	219	270
Phenyl alanine	305	312	307	302	180
Serine	345	356	352	349	
Threonine	166	180	174	169	180
Tryptophan	99	102	118	107	90
Valine	152	164	160	155	270

^{*)} Calculated as mg/g total nitrogen

Bandemer and Evans (18) reported that heating kidney bean caused small losses for most of its amino acids. Myklestadt, Bjoernstadt and Leif (19) showed also that most of the amino acid levels decreased as temperature increased. Taira Harue (20) observed during the preperation of soybean-based foods that Lysine, Cystine, Arginine, Tryptophan and Serine were partially lost during heating. The author stated that heating at high temperature for a long time was the most destructive especially for Lysine and Cystine and that heating with water decreased the loss of Lysine and Arginine but not that of the other amino acids. Boiling the lupin seeds followed soaking them in water resulted in slight losses for most of the amino acids with the exception of Tryptophan which increased slightly.

Toxic factors

Trypsin inhibitors were present in raw lupin seeds as shown in table 4. The level of Trypsin inhibitors was very low (96 units/g sample) as compared with those of other legumes such as kidney bean which contained about 209,000 units/g (21). Trypsin inhibitors were destroyed on boiling lupin seeds for one hour. The levels of Trypsin inhibitors in the raw decorticated seeds were less than those of the whole ones, which means that they are mainly concentrated in the dicotyledon of the seed.

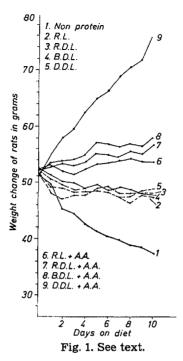
Testing the presence of hemagglutinins, cyanogenetic glucosides and Saponins revealed the absence of these three factors from raw and treated seeds. The presence of Alkaloids was detected in the raw seeds (1.32%). These alkaloids are localised in the dicotyledones. Boiling the seeds for one hour did not eliminate their Alkaloids completely. Boiled seeds contained 1.16 g Alkaloids per 100 g. Debittering the seeds led to the removal of all its Alkaloids.

Feeding experiments

Table 5 shows the net protein utilization of raw and treated seeds. The weight curves of rats fed the different diets of lupin are shown in figure 1. The whole raw seeds had the lowest N.P.U. values, 42. Decorticating or boiling the raw seeds caused no change in the weight of rats and N.P.U. values. Debittering the seeds improved the N.P.U. values from 42 to 56. This improvement may be due to the toxicity of the large amounts of Alkaloids found in the raw seeds which were eliminated by debittering the seeds as mentioned before. Two factors may be the cause of the low N.P.U. values of raw, boiled and debittered seeds. These two factors are,

Treatment	Saponin test	Trypsin inhibitor	Haem- agglutinin	Alkaloids	Cyano- genetic
		U/g	$\widetilde{\mathrm{HU}}/\mathrm{mg}$	g/100	glucoside
R. L.	-ve	96	-ve	1.32	ve
R. D. L.	-ve	101	$-\mathbf{v}\mathbf{e}$	1.40	— ve
B. D. L.	-ve	00	v e	1.16	ve
D. D. L.	$-\mathbf{ve}$	00	ve	0.00	ve

Table 4. Toxic substances present in the raw and treated lupin



the low digestibility coefficient of the protein and their low content of essential amino acids, Lysine and sulfur-containing amino acids. The following table shows the level of Lysine and sulfur-containing amino acids in the lupin seeds as compared to the FAO (22) provisional amino-acid pattern expressed in mg/g nitrogen.

Amino acid	Lupin	FAO pattern
Lysine	215	270
Methionine + Cystine	157	270

Table 5. Net protein utilization of raw, treated, and supplemented lupins

Treatment	NPU value
R. L.	42
R. D. L.	45
B. D. L.	44
D. D. L.	56
R. L. $+$; methionine $+$ lysine	62
R. D. L. $+$ methionine $+$ lysine	60
B. D. L. $+$ methionine $+$ lysine	63
D. D. L. $+$ methionine $+$ lysine	70

= raw lupin R. L.

R. D. L. = raw decorticated lupin

B. D. L. = boiled decorticated

D. D. L. = debittered decorticated seeds

Supplementing the raw, raw decorticated and boiled seeds with the two limiting amino acids Lysine and Methionine raised the N.P.U. values up to 62, 60, and 63, respectively. Also supplementing the debittered seeds with those amino acids raised the N.P.U. value to 70, which is more or less similar to that casein. Similar results were achieved by *Tannous* and his group (16). They showed that growth of rats fed untreated lupin seeds was improved by removing the alkaloid fraction from the seeds or by increasing the level of dietary protein and by supplementing the diets with its limiting amino acids. They ascribed the poor growth observed in rats fed untreated lupin diet to be due to the presence of both alkaloids and the poor quality of dietary proteins.

Taking in consideration that *Lupinus termis* is grown in poor and roughly cultivated lands (23), its use in human dietary of Egyptians will be a good solution for protein shortage.

Summary

- 1. General analysis of the seeds for protein, fats, carbohydrates, fiber and ash contents were carried out and the results were given in g/100 g dry seeds. *Lupinus termis* contained a higher percentage of protein (40.36), 290 mg calcium and 6.5 mg iron.
- 2. All the essential amino acids are present in fair amounts with the exception of sulphur-amino acids and Lysine.
- 3. Cooking the seeds resulted in a slight decrease of most of the amino acids with the exception of Tryptophan and Lysine, which exhibited a slight increase after cooking.
- 4. Of the toxic substances present in the seeds is Trypsin inhibitor, which was destroyed by heat treatment, and alkaloids, which were removed by soaking the seeds three days in running tap water.
- 5. Supplementing the debittered seeds with Lysine and Methionine resulted in an increase in its nutritive value.

Zusammenfassung

- 1. Lupinensamen wurde auf seinen Gehalt an Proteinen, Fetten, Kohlenhydraten, Faser- und Aschestoffen analysiert. Die Ergebnisse wurden in g/100 g für trockenen Samen angegeben. Lupinus termis enthielt einen höheren Prozentsatz an Protein (40,36), 290 mg Calcium und 6,5 mg Eisen.
- 2. Alle essentiellen Aminosäuren mit Ausnahme von schwefelhaltigen Aminosäuren und Lysin sind in angemessener Höhe enthalten.
- 3. Das Kochen der Samen bewirkte eine leichte Verminderung der Aminosäuren mit Ausnahme von Tryptophan und Lysin, wo nach dem Kochen ein leichter Anstieg zu verzeichnen war.
- 4. An toxischen Stoffen sind in den Samen der Trypsin-Inhibitor, der bei Hitzebehandlung zerstört wird, und Alkaloide enthalten. Letztere wurden durch dreitägiges Einweichen der Samen unter laufendem Wasser eliminiert.
- 5. Der Nährwert der entbitterten Samen wurde durch Zusätze von Lysin und Methionin erhöht.

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