JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Nutritional Utilization by the Rat of Diets Based on Lentil (*Lens culinaris*) Seed Meal or Its Fractions

CARMEN CUADRADO,*,† GEORGE GRANT,‡ LUIS A. RUBIO,§ MERCEDES MUZQUIZ,† SUSAN BARDOCZ,‡ AND ARPAD PUSZTAI‡

Departamento de Tecnología de Alimentos, SGIT-INIA, Apartado 8111, 28080 Madrid, Spain; The Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, Scotland, U.K.; and Unidad de Nutrición, Estación Experimental del Zaidin (CSIC), Profesor Albareda 1, 18008 Granada, Spain

The nutritional effects in the rat of raw lentil meal or its fractions have been evaluated in three feeding trials. Growth, gain/feed ratio, apparent N digestibility, and N retention were significantly ($p < 0.05$) reduced by the inclusion of whole lentil meal, dehulled lentil meal, or ethanol-extracted lentil meal as the sole source of protein in the diet. Pure lentil lectin and lectin-depleted albumin proteins had no significant negative effect on nutritional performance. In contrast, growth, gain/feed ratio, protein conversion efficiency, N digestibility, and N retention were significantly ($p < 0.05$) reduced by diets containing lentil globulins or lentil hulls. The poor nutritional quality of raw lentil meal for rats is therefore likely to be primarily due to the combined effects of these two components.

KEYWORDS: Lentils; nutritional utilization; lentil fractions; lentil lectin; rat

INTRODUCTION

Plant proteins are increasingly being used as an alternative to proteins from animal sources in human nutrition. Among plants, legume seeds such as soybean, common beans, chickpeas, lupins, or lentils represent a rich source of proteins, carbohydrates, several water-soluble vitamins, and minerals (*1*). Results from recent investigations provide a biomedical foundation for the beneficial effect of legume intake in experimental animals and in humans (*2*). In this sense, lentils are a rich source of easily available cheap protein in the diet of millions of people in the Mediterranean area, Africa, the Middle East, southern Asia, and South America. The main use of lentil seeds is for direct human consumption as a cooked product (*3*) or following other processing methods (*4*, *5*). However, their use as animal feed is limited due to the low nutritional quality of the raw meal. As with other legumes (*1*−*8*), this is considered to be due in part to the refractory nature of the protein, low sulfur amino acid content, and the presence of heat-stable and heat-labile antinutritive compounds, which must be inactivated or eliminated before lentils can be incorporated into diets at high levels.

Little is, however, actually known on the nutritional properties of lentil constituents (proteins, carbohydrates, ether extract, fiber) in vivo. The aim of the present study was therefore to relate the nutritional performance of growing rats fed diets based on lentil meal as the main protein source to the effects found with fractions obtained from the whole lentil seed meal, including particularly the lectin and lectin-depleted proteins (globulin and albumins). The buffer-insoluble residue, which comprises mainly starch, insoluble nonstarch polysaccharides, and lignin (*6*, *7*), and the seed hulls were also tested.

MATERIALS AND METHODS

Fractionation Procedures. Seeds of *Lens culinaris* var. Magda 20 were obtained from the Instituto Agronómico Provincial, Albacete (Spain). They were ground in a Glen Creston mill fitted with a 1-mm mesh screen. The meal was extracted (1:10 w/v) twice with 70% ice-cold ethanol/water as described by Grant et al. (*9*). The residue (EEM) was washed with acetone and air-dried. The ethanol extracts and the acetone wash were combined, recovered by rotary evaporation, and freeze-dried (EE).

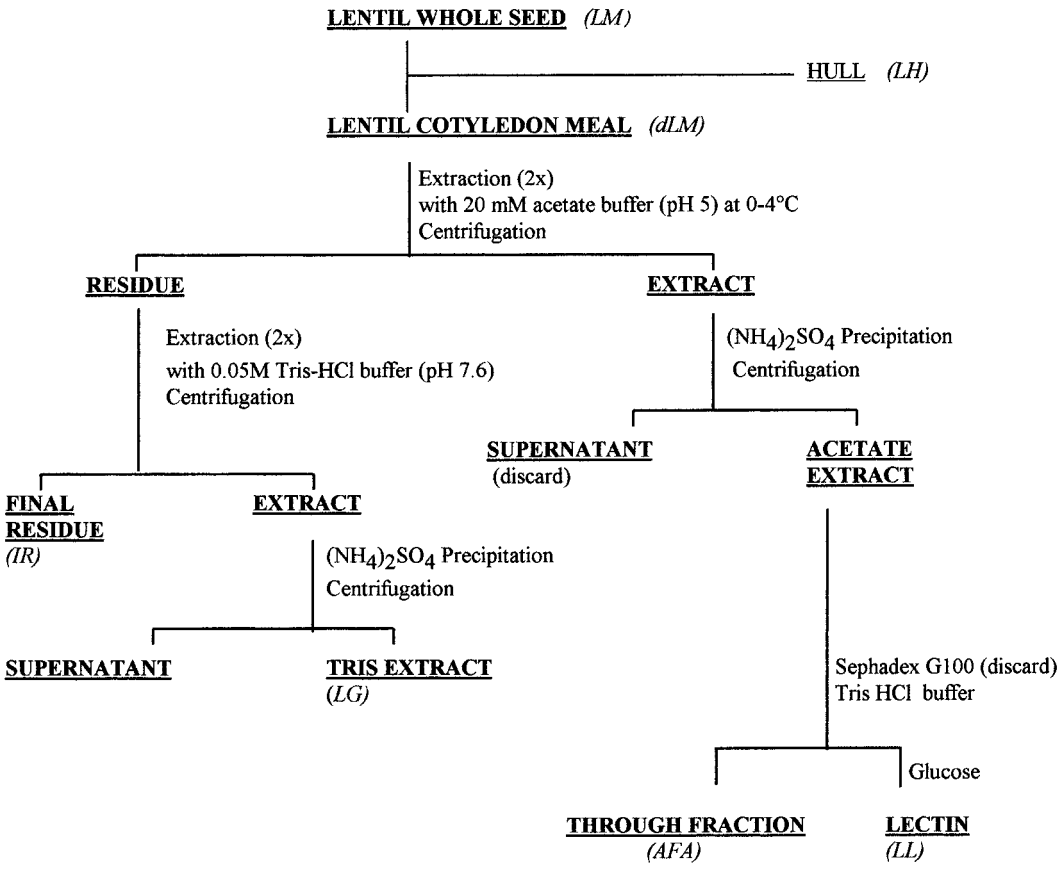
Raw lentil seeds were dehulled in a mill followed by air aspiration, and the hulls (LH) were collected and freeze-dried. The dehulled lentil cotyledons were milled, and the meal (dLM) was extracted as before (*10*) (**Figure 1**), with 20 mM sodium acetate/acetic acid buffer, pH 5.0, and centrifuged; the extract was precipitated with ammonium sulfate (760 g/L) and the sediment extensively dialyzed against water and freeze-dried (albumin-enriched fraction, 21 g/kg of meal). This fraction contained the bulk of the lectin present in lentil meal (*10*). Pure lentil lectin (LL; 0.83 g/kg of meal) was isolated by affinity chromatography of extracted protein on Sephadex G-100 as before (*10*). An agglutinin-depleted albumin protein fraction (AFA; 14 g/kg meal) was also obtained from material unbound to the Sephadex G-100 column.

The acetate-insoluble residue was then extracted with 0.05 M Tris-HCl buffer, pH 7.6, and the supernatant precipitated with ammonium sulfate (760 g/L). The sediment obtained after centrifugation was dialyzed against distilled water and freeze-dried (globulin-enriched fraction, LG; 49 g/kg of meal). The final insoluble residue obtained (IR; 467 g/kg) was also recovered by freeze-drying.

Animals and Diets. Growing male rats (Hooded Lister, Rowett strain), reared and housed in the breeding and experimental unit of the Rowett Research Institute, were used in this study. They were weaned at 19 days of age and given free access to a stock diet (Labsure, Manea,

† SGIT-INIA.
‡ The Rowett Research Institute.
§ CSIC.

10.1021/jf020014j CCC: $22.00
Published on Web 06/17/2002



	Weight ($g.kg^{-1}$ dm)	N content ($g.kg^{-1}$ dm)
Lentil whole seeds (LM)	1000	45
Hulls (LH)	200	5.1
Lentil cotyledon meal (dLM)	800	53
Acetate extract	21.25	nd
Agglutinin free Albumins (AFA)	14.24	138
Lentil lectin (LL)	0.83	nd
Tris extract (LG)	49.24	127
Final residue (IR)	467	13

nd: not determined

Figure 1. Lentil fractionation procedures and fraction yields.

U.K.) for 10 days, followed by the high-quality control diet (LA; **Table 1**) for 3 days to ensure their adaptation to the experimental conditions. Five rats per group, matched by weight (82 ± 2 g in experiments 1 and 2 and 85 ± 1 in experiment 3), were housed individually in metabolism cages. Water was freely available at all times. The animals were weighed daily. Urine and feces were collected daily and stored at −20 °C until required. After a 10-day experimental period, the rats were killed by halothane (Rhone Merieux, Essex, U.K.) overdose. The carcass and fecal samples were freeze-dried and ground with a coffee grinder. Total nitrogen was estimated by a semiautomated macro-Kjeldahl method (*11*).

The Rowett Research Institute is licensed under the U.K. Animals (Scientific Procedures) Act 1986. The ethical review committee and the animal welfare unit of the Institute and the appropriate governmental inspectorate monitor and review all animal studies. All management and experimental procedures were approved and done in strict accordance with the requirements of the U.K. Animals (Scientific Procedures) Act 1986 by staff personally licensed to carry out such procedures.

Diets in the experiments (**Table 1**) were formulated to contain the same amount of total proteins (100 g/kg) and, where appropriate, were supplemented with amino acids to target requirements for rats (*9*). Crude protein was calculated as N × 6.25 for lactalbumin and as N × 5.4 for lentil protein (*12*). All rats were fed at 6 g of diet/day, given as two feeds over the day. This was close to the free daily intake of rats given raw *L. culinaris* var. Magda 20 as their sole source (100 g/kg) of dietary protein but was above that necessary (5 g/day) to meet the minimum energy, mineral, and vitamin requirements of rats (*9*). In experiment 1 the diets used were lactalbumin control (LA), lentil seed meal (LM), ethanol-extracted meal (EEM), and the ethanol extract (EE). In

Table 1. Composition (Grams per Kilogram) of Diets

	diet[a]									
	LA	LM	EEM	EE	dLM	LH	IR	LG	AFA	LL
LM		412								
EEM			312							
EE				92						
dLM					349					
hulls						105				
IR							211			
LG								145		
AFA									86.2	
LL										1.6
LA	120			120		116	102		43	118
maize starch	380	88	188	288	153	280	189	356	371	380
potato starch	100	100	100	100	100	100	100	100	100	100
glucose	150	150	150	150	150	150	150	150	150	150
corn oil	150	150	150	150	150	150	150	150	150	150
vitamins/minerals[b]	100	100	100	100	100	100	100	100	100	100
silicic acid	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
added amino acids[c]										
L-arginine		0.5	0.5		0.5		0.1	0.5	0.3	
L-histidine		0.9	1.0		1.0		0.1	1.0	0.6	
L-lysine		1.0	1.0		1.0		0.1	1.0	0.7	
L-tyrosine		2.7	3.0		2.7	0.2	0.4	2.7	1.7	
L-tryptophan		1.0	1.0		1.0		0.1	1.0	0.6	
L-methionine		1.5	2.0		1.6	0.1	0.3	1.6	1.0	
L-threonine		1.1	1.3		1.1	0.1	0.2	1.1	0.7	
L-isoleucine		0.3	0.4		0.4			0.4	0.2	
L-valine		2.0	2.1		2.0	0.1	0.4	2.0	1.3	

[a] LA, lactalbumin control diet; LM, whole lentil meal; EEM, ethanol-extracted meal; EE, ethanol extract; dLM, dehulled lentil meal; LH, lentil hulls; IR, insoluble lentil residue; LG, lentil globulins, AFA, agglutinin-free albumins; LL, lentil lectin. [b] Vitamin and mineral mixes as per Grant et al. (*5*). [c] Essential amino acids added to diets according to composition of meal and fractions to reach LA values.

Table 2. Growth, Performance, Body Composition, and Excretion of Rats Fed Diets Containing Lentil Seed Meal or Its Fractions for 10 Days (Experiment 1)[a]

	control (LA)	lentil meal (LM)	alcohol extracted meal (EEM)	alcohol extract (EE)
initial body wt (g)	82.3 ± 1.1a	82.9 ± 2.1a	81.1 ± 0.9a	80.2 ± 1.5a
final body wt (g)	94.6 ± 1.5a	80.9 ± 1.5b	82.0 ± 1.7b	93.1 ± 2.3a
wt gain (g)	12.3 ± 0.6a	0.7 ± 1.5b	0.9 ± 0.8b	10.2 ± 0.9a
gain:feed (g/g)	0.21 ± 0.02a	0.01 ± 0.04b	0.02 ± 0.03b	0.19 ± 0.03a
protein conversion efficiency (g/g)	2.1 ± 0.2a	0.1 ± 0.3b	0.2 ± 0.3b	1.9 ± 0.3a
body composition				
dry body wt (g)	26.5 ± 0.6a	23.4 ± 0.5b	23.6 ± 0.6b	26.5 ± 1.3a
body N (g)	2.72 ± 0.08a	2.33 ± 0.11b	2.25 ± 0.07b	2.65 ± 0.10a
excretion				
fecal dry wt (g)	4.0 ± 0.7a	9.1 ± 1.7b	8.1 ± 1.5b	4.1 ± 0.9a
fecal N (mg)	177 ± 15a	381 ± 40b	368 ± 35b	195 ± 10a
urine N (mg)	155 ± 15a	399 ± 35b	400 ± 33b	160 ± 12a

[a] Values in a row with different letters differ significantly ($p \leq 0.05$).

experiment 2 the diets were LA, LM, dehulled meal (dLM), hulls (LH), insoluble residue (IR), and the globulin-enriched proteins (LG). In experiment 3 the diets were LA, LM, lectin (LL), and agglutinin (lectin)-depleted albumin proteins (AFA). LA, LM, EEM, dLM, and LG were incorporated in the diet as the sole (100 g/kg) sources of dietary protein. IR, EE, LH, and AFA were added to the diets in the same proportion as in the LM diet. The amount of lentil lectin (LL) added in diet LL was ~5-fold the concentration in the LM diet. When lentil components constituted only part of the protein in the diet, supplementary lactalbumin was added to reach a final protein concentration of 100 g/kg.

Statistical Analysis. Data were assessed by one-way analysis of variance in combination with the appropriate multiple-comparison test using the Instat Statistical Software package (GraphPad Software Inc., San Diego, CA).

RESULTS

The growth of rats fed raw fully supplemented lentil meal as their sole protein source (diet LM) for 10 days was significantly depressed in comparison with control rats fed the same amount daily of a high-quality diet (diet LA) (**Tables 2−4**). The final body weight of rats fed whole lentil meal for 10 days did not differ significantly from their initial body weight. Animals fed on LM excreted high amounts N through feces and urine and had significantly ($p < 0.05$) lower gain/feed ratios and body N than controls (**Tables 2−5**). Urinary N excretion by these rats was particularly high. As a result, the apparent digestibility of N and the retention of absorbed N were lower than controls ($p < 0.05$) (**Table 5**).

The growth, dry body weight, and body composition of animals fed either on whole seed meal (LM) or on extracted seed meal (EEM) did not differ significantly (**Table 2**). Furthermore, the nutritional performance of rats given lactalbumin-based diets containing the ethanol extract of lentil meal (EE) was not different from that of controls (LA).

The weight gain of rats fed diets containing dLM, LH, and LG was lower than that of controls but similar to those for LM-

Table 3. Growth, Performance, Body Composition, and Excretion of Rats Fed Diets Containing Lentil Seed Meal or Its Fractions for 10 Days (Experiment 2)[a]

	control (LA)	lentil meal (LM)	dehulled meal (dLM)	hulls (LH)	insoluble residue (IR)	globulins (LG)
initial body wt (g)	83.0 ± 1.3a	83.2 ± 1.0a	83.3 ± 0.9a	82.9 ± 1.2a	81.9 ± 2.1a	81.7 ± 1.5a
final body wt (g)	97.2 ± 1.3a	85.5 ± 1.0b	85.7 ± 1.0b	87.0 ± 2.2b	94.8 ± 1.6a	83.8 ± 1.9b
wt gain (g)	13.1 ± 0.6a	2.3 ± 0.5b	2.4 ± 0.7b	4.1 ± 1.0b	13.0 ± 1.1a	2.0 ± 1.0b
gain:feed (g/g)	0.23 ± 0.02a	0.04 ± 0.01b	0.04 ± 0.01b	0.07 ± 0.02c	0.22 ± 0.02a	0.03 ± 0.02b
protein conversion efficiency (g/g)	2.3 ± 0.2a	0.4 ± 0.1b	0.4 ± 0.1b	0.7 ± 0.2c	2.2 ± 0.2a	0.3 ± 0.2b
body composition						
dry body wt (g)	25.8 ± 0.7a	22.7 ± 0.8b	23.3 ± 0.7b	23.1 ± 0.5b	26.3 ± 1.2a	23.9 ± 0.6b
body N (g)	2.64 ± 0.08a	2.19 ± 0.09b	2.19 ± 0.07b	2.27 ± 0.04b	2.71 ± 0.14a	2.31 ± 0.07b
excretion						
fecal dry wt (g)	4.7 ± 0.9a	9.4 ± 1.1b	8.5 ± 1.5b	6.9 ± 1.7ab	7.1 ± 1.2ab	5.5 ± 0.9ab
fecal N (mg)	196 ± 11a	377 ± 35b	360 ± 25b	399 ± 45b	234 ± 42a	366 ± 35b
urine N (mg)	166 ± 11a	420 ± 40b	405 ± 42b	308 ± 45c	188 ± 32a	351 ± 35bc

[a] For each experiment values in a row with different letters differ significantly ($p \leq 0.05$).

Table 4. Growth, Performance, Body Composition, and Excretion of Rats Fed Diets Containing Lentil Seed Meal or Its Fractions for 10 Days (Experiment 3)[a]

	control (LA)	lentil meal (LM)	lectin (LL)	agglutinin-free albumins (AFA)
initial body wt (g)	85.3 ± 0.9a	85.0 ± 0.8a	85.2 ± 1.5a	85.3 ± 1.2a
final body wt (g)	99.7 ± 1.0a	84.7 ± 0.8b	99.1 ± 2.1a	99.2 ± 2.2a
weight gain (g)	14.4 ± 0.5a	–0.2 ± 0.5b	13.9 ± 0.6a	10.2 ± 0.9a
gain:feed (g/g)	0.24 ± 0.02a	–0.01 ± 0.01b	0.23 ± 0.02a	0.23 ± 0.02a
protein conversion efficiency (g/g)	2.4 ± 0.2a	–0.03 ± 0.12b	2.3 ± 0.2a	2.3 ± 0.2a
body composition				
dry body wt (g)	28.6 ± 1.3a	24.9 ± 0.3b	27.8 ± 0.8a	28.2 ± 0.8a
body N (g)	2.93 ± 0.05a	2.44 ± 0.06b	2.86 ± 0.06a	2.91 ± 0.07a
excretion				
fecal dry wt (g)	4.3 ± 0.9a	8.9 ± 1.0b	3.9 ± 0.9a	4.5 ± 0.7a
fecal N (mg)	186 ± 8a	340 ± 30b	190 ± 15a	199 ± 20a
urine N (mg)	155 ± 10a	470 ± 40b	175 ± 25a	166 ± 25a

[a] Values in a row with different letters differ significantly ($p \leq 0.05$).

Table 5. Apparent Fecal Digestibility of N and Retention of Absorbed N by Rats Fed Diets Containing Lentil Meal or Its Fractions[a]

		apparent fecal N digestibility (%)	apparent N retention (%)
control	LA	80.2 ± 1.3a	78.8 ± 1.3a
whole meal	LM	62.3 ± 1.7b	29.9 ± 2.5b
dehulled meal	dLM	62.1 ± 3.3b	31.4 ± 4.0b
hulls	LH	58.9 ± 2.6b	46.1 ± 6.0c
insoluble residue	IR	75.9 ± 3.2c	74.4 ± 4.1a
globulins	LG	62.0 ± 3.5b	42.6 ± 5.1c
lectin-free albumins	AFA	78.9 ± 2.1ac	77.9 ± 4.1a
lectin	LL	80.8 ± 1.5a	78.1 ± 3.2a

[a] Values in a column with different letters differ significantly ($p \leq 0.05$). Apparent fecal N digestibility was calculated as [(intake N – fecal N)/intake N] × 100. Apparent N retention was calculated as [(absorbed N – urinary N)/absorbed N] × 100. Absorbed N was calculated as intake N – fecal N.

fed animals (**Table 3**). As with LM, rats fed dLM, and LG excreted high amounts of N through the feces and particularly in the urine, leading to low apparent N digestibility and retention of absorbed N and protein conversion efficiency values (**Tables 3** and **5**). Fecal and urinary N excretion was also elevated in rats fed diets containing lentil hulls (LH). However, in these rats urinary N excretion was not as high as that for rats fed LM, dLM, or LG, although fecal N outputs were similar for these last three (**Tables 3** and **5**). Protein conversion efficiency and gain/feed values obtained with diet LH were therefore lower than control (LA) values but significantly higher ($p < 0.05$) than those for the LM, dLM, and LG diets. In contrast, fecal and urinary N excretion, protein conversion values, weight gains, and final dry body weights for rats fed IR were similar to those for controls (**Tables 3** and **5**).

Lentil lectin (LL) and lectin-depleted albumins (AFA) did not appear to significantly affect the metabolism of rats (**Tables 4** and **5**). Thus, weight gain, fecal and urinary N excretion, protein conversion efficiency, gain/feed ratio, dry body weights, and composition for rats fed these diets were similar to those for controls given high-quality control diet.

DISCUSSION

Raw lentil seed meal included in the diet as the only source of protein (100 g/kg) did not meet the nutritional requirements of growing rats, even when the diet was fully supplemented with essential amino acids in agreement with previous work (*3*). However, the nature of the factors responsible for impairing the appropriate nutritional utilization of lentil meal by the growing rat remains unclear.

The low nutritional value of whole lentil meal was not improved by removal of low molecular weight substances (tannins and saponins) and ethanol-soluble carbohydrates by aqueous ethanol extraction. Furthermore, inclusion of the ethanol extract in the control diet had no negative effect. This contrasts with the beneficial effects of aqueous ethanol extraction on the nutritional value of soybean (*9*). Also, even though some lectins can be deleterious to animals in vivo (*13*), the low nutritional value of the lentil seed meal did not appear to be due to its lectin content, because inclusion of 5 times as much lectin in lactalbumin-based diets as found in LM diets did not influence

the growth or protein utilization of the rats. Thus, lentil lectin, like many other mannose/glucose specific lectins (*7*, *13*, *14*), may in most circumstances have little or no effect on the metabolism of animals. In addition, the insoluble residue from dehulled lentils (IR) had little or no deleterious effect on the nutritional performance of rats. This suggests, as previously found with faba beans and chickpeas (*7*, *9*, *22*), that starch and insoluble fiber (cell wall materials) of lentils are well utilized and have no significant adverse effects when included in diets for rats.

Apparent N digestibility and N retention were significantly reduced and the growth of rats was limited when the globulin fraction (LG) was included in the diet as the sole protein source. This suggests that poor utilization of the globulin proteins may, in part, be responsible for the low nutritional quality of lentil meal for rats. Recent studies carried out with purified lupin, faba bean, soybean, and chickpea proteins concluded that the main reasons for the low nutritional value of these legume meals might be related in some unknown way to the chemical structure of their globulin proteins and the adverse effects of these proteins or digestion products of them on growth and nitrogen metabolism, rather than to the presence of antinutritional factors (*7*, *9*, *15*, *16*, *19*, *22*). The low fecal apparent N digestibility of lentil storage proteins observed in the present study contrasts with some previous results (*16*, *19*) with other legume proteins in which ileal digestibility values were high and comparable to those of control proteins. The reasons for this difference between ileal and fecal results are unclear but may be linked to the presence in the globulin-enriched protein fraction of tannins, which lower protein digestibility (*17*), or soluble fiber in the lentil meal, which by interacting with the seed globulins may cause high N excretion through the feces (*18*). Alternatively, the globulins may have stimulated secretion of endogenous protein in the intestine. The increased endogenous secretion might be related to abnormal local immune reactions in the intestine (*17*). This can lead to high excretion of N in the feces, even though the globulin itself may be completely broken down before digesta moves into the large intestine (*19*−*21*).

Urinary N excretion by rats fed lentil globulins (LG) was greatly elevated compared to controls and as high as for those fed whole lentil meal. This was consistent with previous findings with lupin, faba bean, and soybean globulins (*19*). It may indicate that, as with these other globulins (*19*), the inability to effectively retain absorbed N was a major factor limiting the nutritional performance of rats fed LG. This was unlikely to be due directly to amino acid imbalances because the diet was supplemented with essential amino acids up to the requirements for rats. Lentil globulins or breakdown products derived from them may have modulated or interfered with intestinal or systemic metabolism and, as a result, limited retention of N (*19*−*21*).

The presence of lentil hulls in the diet adversely affected utilization of a high-quality protein (lactalbumin) in vivo. In addition, the hulls also appeared to prevent ready solubilization and extraction of globulins from the whole seed in vitro (185 g of protein solubilized/kg of dehulled meal as compared to 77.5 g/kg from whole meal; data not shown). The inefficient utilization of LM by rats may therefore have been primarily due to the poor utilization of lentil globulin proteins in combination with the adverse effects of factors from hulls on the availability of dietary proteins and/or other nutritional components of the diet. These factors are likely to be linked to fiber fractions and/or tannins, which have been shown to affect protein digestibility and excretion of endogenous protein (*23*). The involvement of other components in the seed meal cannot, however, be completely excluded at present because neither LG nor LH reduced apparent N retention by as much as did whole lentil meal.

ABBREVIATIONS USED

LA, lactalbumin control diet; LM, whole lentil meal; EEM, ethanol-extracted meal; EE, ethanol extract; dLM, dehulled lentil meal; LH, lentil hulls; IR, insoluble lentil residue; LG, lentil globulins; AFA, agglutinin-free albumins; LL, lentil lectin.

LITERATURE CITED

(1) Friedman, M. Nutritional value of proteins from different food sources. A review. *J. Agric. Food Chem.* **1996**, *44*, 6−29.

(2) Zulet, M. A.; Martinez, A. Mediterranean diet: legumes and cholesterolemia. *Revista Chilena de Nutrición* **2001**, *28*, 312−320.

(3) Savage, G. P. The composition and nutritive value of lentils (*Lens culinaris). Nutr. Abstr. Rev.* **1988**, *58*, 321−343.

(4) Cuadrado, C.; Ayet, G.; Robredo, L. M.; Tabera, J.; Villa, R.; Burbano, C.; Muzquiz, M. Effect of natural fermentation on phytates content of lentils. *Z. Lebensm. Unters. Forsch.* **1996**, *203*, 268−271.

(5) Ayet, G.; Burbano, C.; Cuadrado, C.; de la Cuadra, C.; Robredo, L. M.; Castaño, A.; Muzquiz, M.; Osagie, A. Effect of germination, under different enviromental conditions, on saponins, phytic acid and tannins in lentils (*Lens culinaris*). *J. Sci. Food Agric.* **1997**, *74*, 273−279.

(6) Rubio, L. A.; Grant, G.; Bardocz, S.; Dewey, P.; Pusztai, A. Nutritional response of growing rats to faba beans (*Vicia faba* L., minor) and faba bean fractions. *Br. J. Nutr.* **1991**, *66*, 533−542.

(7) Rubio, L. A.; Grant, G.; Scislowski, P. W. O.; Brown, D.; Bardocz, S.; Pusztai, A. The utilization of lupin (*Lupinus angustifolius)* and faba bean (*Vicia faba*) globulins by rats is poorer than of soybean globulins or lactalbumin but the nutritional value of lupin seed meal is lower only than that of lactalbumin. *J. Nutr.* **1995**, *125*, 2145−2155.

(8) Sanz, M. A.; Blázquez, I.; Sierra, I.; Medrano, M. A.; Frias, J.; Vidal-Valverde, C.; Hernández, A. Nutritional evaluation of ethanol-extracted lentil flours. *J. Agric. Food Chem.* **2001**, *49*, 1854−1860.

(9) Grant, G.; McKenzie, N. H.; Watt, W. B.; Stewart, J. C.; Dorward, P. M.; Pusztai, A. Nutritional evaluation of soya beans (*Glycine max*). *J. Sci. Food Agric.* **1986**, *37*, 1001−1010.

(10) Cuadrado, C.; Burbano, C.; Gelencser, E.; Grant, G.; Pusztai, A. Large scale purification of lectin from lentil seeds and determination of the lectin content in processed lentils. In *Cost 98—Effects of Antinutrients on the Nutritional Value of Legume Diets*; Bardocz, S., Muzquiz, M., Pusztai, A., Eds.; Office for Official Publications of the European Communities: Luxembourg, 1997; Vol. IV, pp 82−88.

(11) Davidson, J.; Mathieson, J.; Boyne, A. W. The use of automation in determining nitrogen by the Kjeldahl method, with the final calculations by computer. *Analyst* **1970**, *95*, 181−193.

(12) Mossé, J. Nitrogen to protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed protein content. *J. Agric. Food Chem.* **1990**, *38*, 18−24.

(13) Grant, G. Plant lectins. In *Secondary Plant Products. Antinutritional and Beneficial Actions in Animal Feeding*; Caygill, J. C., Mueller-Harvey, I. Eds.; Nottingham University Press: Nottingham, U.K., 1999; pp 87−110.

(14) Pusztai, A.; Ewen, S. W. B.; Grant, G.; Peumans, W. J.; van Damme, E. J. M.; Rubio, L.; Bardocz, S. The relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. *Digestion* **1990**, *46* (Suppl. 2), 308−316.

(15) Rubio, L. A.; Grant, G.; Daguid, T.; Brown, D.; Pusztai, A. Organ relative weights and plasma amino acid concentrations in rats fed diets based in legume (faba bean, lupin, chickpea, soybean) seed meals or their fractions. *J. Sci. Food Agric.* **1999**, *7*, 187−194.

(16) Rubio, L. A.; Grant, G.; Caballé, C.; Martinez-Aragón, A.; Pusztai, A. High in vivo (rat) digestibility of faba bean (*V. faba*), lupin (*L. angustifolius*) and soybean (*G. max*) globulins. *J. Sci. Food Agric.* **1994**, *66*, 289−292.

(17) Lalles, J. P.; Jansman, A. J. M. Recent progress in the understanding of the mode of action and effects of antinutritional factors from legume seeds in non-ruminant farm animals. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds and Rapeseed*; Jansman, A. J. M., Hill, G. D., Huisman, J., van der Poel, A. F. B., Eds.; Wageningen Pers: Wageningen, The Netherlands, 1998; pp 219−232.

(18) Mason, V. C. Metabolism of nitrogenous compounds in the large intestine. *Proc. Nutr. Soc.* **1984**, *43*, 45−53.

(19) Rubio, L. A. Physiological effects of legume storage proteins. *Nutr. Abstr. Rev. (Ser. A)* **2000**, *70*, 197−204.

(20) Santoro, L. G.; Grant, G.; Pusztai, A. Effects of short-term feeding of rats with a highly purified phaseolin preparation. *Plant Foods Hum. Nutr.* **1997**, *51*, 61−70.

(21) Santoro, L. G.; Grant, G.; Pusztai, A. In vivo degradation and stimulating effect of phaseolin on nitrogen secretion in rats. *Plant Foods Hum. Nutr.* **1999**, *53*, 223−236.

(22) Rubio, L. A.; Grant, G.; Daguid, T.; Brown, D.; Bardocz, S.; Pusztai, A. The nutritional utilization by rats of chickpea (*Cicer arietinum*) meal and its isolated globulin proteins is poorer than that of defatted soybean or lactalbumin. *J. Nutr.* **1998**, *128*, 1042−1047.

(23) Jansman, A. J. M.; Verstegen, M. W. A.; Huisman, J.; van den Berg, J. W. O. Effects of hulls of faba beans (*Vicia faba* L.) with a low or high content of condensed tannins on the apparent ileal and fecal digestibility of nutrients and the excretion of endogenous protein in ileal digesta and fecaes of pigs. *J. Anim. Sci.* **1995**, *73*, 118−127.

Received for review January 7, 2002. Revised manuscript received April 24, 2002. Accepted April 24, 2002. This work was supported by the Spanish MAPA (INIA Project SC93-162) and SEERAD and was part of COST98. C.C. gratefully acknowledges the Spanish Ministerio de Educacion y Ciencia for the award of a postdoctoral grant and the EU/COST for Short-Term Scientific Missions.

JF020014J