Nutritional evaluation of the germ meal and its protein isolate obtained from the carob seed *(Ceratonia siliqua)* in the rat

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1. Evaluation of the germ meal (CGM) of carob seed (*Ceratonia siliqua*) and its protein isolate was carried out with weanling rats. Comparisons were made with casein, soya-bean meal, whole defatted egg and a soya-bean protein isolate (Promine-D) as protein sources. The growth-promoting effects and certain biological indices were evaluated using the protein efficiency ratio (PER), biological value (BV) and net protein utilization (NPU) bioassay procedures.

2. The unsupplemented CGM had a PER of 1·66±0·09 and an NPU of 0·58±0·013. Addition of DL-methionine at 4, 8 and 12 g/kg diet resulted in a PER of 1·95±0·11, 2·01±0·11 and 1·90±0·11 respectively. The corresponding BV values were 0·80±0·003, 0·78± 0·015 and 0·74±0·011, and those for NPU 0·69±0·013, 0·66±0·026 and 0·63±0·020 respectively. The addition of amino acids improved the PER (2·24–2·59), BV (0·78–0·79) and NPU (0·71–0·73) values.

3. The BV and NPU assays for the unsupplemented carob germ isolate were low (BV 0·36±0·016, NPU 0·35±0·015). Supplementation with amino acids resulted in a positive increase with values of 0·66±0·013 and 0·64±0·013 for BV and NPU respectively.

The nutritional potential of certain agricultural by-products has not been adequately studied, a fact that limits their utilization as animal food ingredients. The shortage and high prices paid for protein concentrates in livestock feeding has stimulated nutritional and economic feasibility studies of by-products and wastes as sources of protein. It appears therefore that the resources in such products need to be further exploited and thoroughly evaluated nutritionally.

Carob germ meal (CGM) is a by-product obtained from the germ of the carob seed (*Ceratonia siliqua*) after the separation of gums and the fibrous coating of the seed. The milled germ has a uniform consistency and is a light greyish colour. It provides in the dry state as fed, approximately 955 g dry matter and 426 g crude protein (nitrogen×6·25)/kg. The current annual production of this by-product in Greece is approximately 2000 t and is likely to increase.

The literature contains only one report by Ferreira (1964) of the evaluation of CGM protein. The CP content varies from 488 to 507 g/kg and biological indices (biological value (BV) 0·51, protein efficiency ratio (PER) 1·20) have been reported for the rat.

In the present study well-established rat bioassay procedures were used to assess protein quality and obtain information on the nutritional value of CGM. An attempt has also been made to prepare a protein isolate from the CGM and evaluate in a comparative study its performance in the rat. The biological indices measured were BV, net protein utilization (NPU), PER and growth response of the rats to different levels of amino acid supplementation.

EXPERIMENTAL

Analytical procedures

Standard methods for moisture, CP, diethyl ether extract, crude fibre and ash were those described by the Association of Official Analytical Chemists (1970). Gross energy was determined in an adiabatic bomb calorimeter (Gallenkamp Co.). Total phenolic compounds

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Table 1. *Composition of carob* (Ceratonia siliqua) *germ meal (CGM)* and CGM protein isolate*

(Mean values of two determinations)

Analytical composition	CGM (g/kg)	CGM protein isolate (g/kg)
Moisture	45·0	14·0
Total nitrogen	68·2	141·9
Diethyl ether extract	56·6	—
Crude fibre	49·9	—
N-free extract	374·6	—
Total ash	47·7	—
Total energy (MJ/kg)	18·8	19·8
Total phenolic compounds	4·3	3·0
Amino acid profile (g/16 g N)		
Aspartic acid	7·47	7·00
Threonine	3·16	2·51
Serine	4·23	4·02
Glutamic acid	25·26	27·80
Proline	4·31	1·87
Glycine	4·43	4·23
Alanine	3·84	2·96
Valine	4·06	2·16
Isoleucine	3·13	2·15
Leucine	5·78	5·86
Tyrosine	2·78	2·00
Phenylalanine	2·61	2·30
Lysine	5·16	4·51
Histidine	2·29	2·29
Arginine	11·55	12·40
Methionine	1·16	0·47
Cystine	1·63	0·48
FDNB, fluorodinitrobenzene reactive lysine	4·70	3·47

* The part remaining after removal of gums and seed coat.

were extracted by boiling 0·5 g CGM in 100 ml distilled water for 30 min and estimated in the filtrate by the Folin-Denis colorimetric method as described in the Association of Official Analytical Chemists (1970). The proximate composition of CGM and its protein isolate and the amino acid profile are given in Table 1.

Amino acids were determined by column chromatography using a Spinco Beckman 120-C automatic amino acid analyzer (Beckman Instruments, Palo Alto, Calif. USA). The samples were weighed and 6 M-hydrochloric acid added in glass-tubes frozen in liquid N_2 and vacuum-sealed. The ratio of sample to hydrochloric acid was 1 mg sample: 1 ml acid and the hydrolysis continued for 24 h at 110°. The hydrolysate was then filtered and the acid removed by evaporation under reduced pressure. The residue was taken up in 6 ml 0·2 M-sodium citrate buffer, pH 2·2. For the determination of sulphur-amino acids the samples were first oxidized with performic acid for 18 h according to the method of Lewis (1966) before acid hydrolysis. Fluorodinitro-benzene-reactive lysine was determined by the method of Carpenter (1960) as modified by Booth (1971). All analyses were replicated and accepted with a deviation from the mean value not more than 5%.

Preparation of the protein isolate

A measured quantity (75 g) of the CGM was used, suspended in 1 l water and the pH adjusted to 1·5 with hydrochloric acid. The suspension was stirred for 1 h at room temperature and the supernatant fraction was separated after centrifugation (4000 *g* for 20 min).

Table 2. *Composition (g/kg) of experimental diets containing the stock protein-free, whole defatted egg, carob* (Ceratonia siliqua) *germ meal (CGM) and the CGM protein isolate*

		PER and NPU tests		N-balance tests				
Ingredient	Stock protein-free*	Egg*	CGM	CGM	CGM protein isolate	Casein	Soya bean	Promine-D
Maize starch	660	490	360					
Sucrose	200	250	250					
Cellulose	50	50	50					
Maize oil	50	50	50	Stock protein-free diet added to make up 1 kg diet				
Mineral salts†	30	30	30					
Vitamin supplement†	10	10	10					
Egg powder‡	—	120	—					
CGM	—	—	250	208	—	—	—	—
CGM protein isolate	—	—	—	—	105	—	—	—
Casein	—	—	—	—	—	101	—	—
Soya-bean	—	—	—	—	—	—	188	—
Promine-D	—	—	—	—	—	—	—	97
Total	1000	1000	1000	1000	1000	1000	1000	1000
Amino acid supplement‖	—	—	1, 2, 3, 4, 5		6	—	—	—
Total nitrogen (by analysis)	0·76	17·00	16·50	14·15	14·17	14·29	14·11	14·03

* Also used in the N-balance test.
† Drouliscos & Bowland (1969).
‡ Egg powder (40 g/kg) was included in the stock protein-free diet for the determination of the metabolic and endogenous N in the N-balance tests.
‖ Supplements (g/kg) 1, 4 DL-methionine; 2, 8 DL-methionine; 3, 12 DL-methionine; 4, 4 DL-methionine; 5 L-Lysine; 5, 3 L-Lysine, 4·5 DL-methionine, 3 DL-phenylalanine, 0·5 DL-histidine, 3 DL-isoleucine, 3 DL-leucine, 2·5 DL-valine, 0·5 DL-tryptophan, 2 DL-threonine; 6, 4·5 L-lysine 3·0 DL-threonine, 4·0 DL-valine, 2·0 DL-isoleucine, 6·0 DL-phenylalanine, 4·5 DL-methionine.

The residue was washed once with 500 ml water and the supernatant fraction collected after centrifugation. Protein in the combined supernatant fractions was precipitated at pH 4·5. After washing with water the protein was separated by centrifugation and freeze dried.

Animals

The rats used for the bioassays were of both sexes and of the Hooded strain weighing 45–50 g for PER and NPU (carcass analysis) tests and 75–80 g for the N balance study. The number of rats varied from six to ten for PER, a total of eight rats (four in each of the duplicate tests) for the NPU and four to five for the N balance. For each of the balance experiments the rats were placed in individual cages equipped for the separation of faeces and urine and the measurement of food intake. The experimental period lasted for 10 d (5 d preliminary and 5 d collection). For NPU determination the rats were caged in groups of four and the experimental period lasted for 10 d preceded by a 5 d preliminary period. In the PER experiment the rats were caged individually and the experiment lasted for 28 d. Food was offered *ad lib.* for PER and NPU tests and a premeasured quantity of food calculated to provide 150 mg N and 10 g dry matter/rat per d was offered to each rat for the N balance tests as described by Eggum (1973). All animals were maintained under controlled temperature ($21 \pm 1°$) and humidity (55%) conditions.

Diets

The composition of the experimental diets is given in Table 2. The stock protein-free diet was mixed with the test protein to provide the required N and DM content for the nitrogen balance experiments.

Nutritional indices

PER was calculated from values for body-weight gain and CP intake over a 28 d period with a diet providing 16 g N/kg. PER was calculated as body-weight gain/unit CP intake. In each case the figures of CP intake used for the calculation of PER, N balance and NPU were based on actual nitrogen analysis of the test diets. NPU was calculated from information on carcass composition with a protein-free and a test diet.

The procedure followed was as described by Bender & Miller (1953) and Miller & Bender (1955). The N balance trials were carried out according to the procedures followed by Eggum (1973). Metabolic and endogenous N were determined separately with a protein-free diet in several replicates (four runs with 21 rats) and the mean values were used in the determination of BV and true digestibilities (TD).

RESULTS AND DISCUSSION

Analysis of CGM (Table 1) indicated that the CP content of 426 g/kg is at a level comparable to that of the soya-bean meal (SOM-45, 457 g/kg) (Drouliscos, 1976) and certain other protein-rich vegetable concentrates.

The amino acid profile of CGM (Table 1) suggests that methionine and cystine are first limiting. The remaining amino acids were also present at levels that were somewhat lower than those recommended in the FAO/WHO pattern (Table 3). In comparison with the FAO/WHO amino acid pattern CGM and CGM protein isolate contain insufficient leucine, isoleucine, threonine and valine. Arginine is exceptionally high in CGM (11·55 g/16 g N) and CGM protein isolate (12·40 g/16 g N). A high arginine content (11·24 g/16 g N) was also reported by Ferreira (1964). When the values of essential amino acids (Table 4) were expressed as essential amino acid content:N content, the value for CGM (2·56) was higher than that of the CGM protein isolate (2·18) but lower than those of the soya-bean protein isolate (Promine-D) (2·68), SOM-45 (2·80), casein (3·28) and whole defatted egg

Table 3. *Essential amino acid (AA) pattern (gAA/16 g N) and amounts of each AA provided by carob* (Ceratonia siliqua) *germ meal (CGM)* and CGM protein isolate*

	Amino acid pattern FAO/WHO (1973)	Protein source			
		CGM		Isolate	
Phenylalanine }	6·0†	5·4†		4·3†	
Tyrosine }					
Isoleucine	4·0	3·1		2·2	
Leucine	7·0	5·8		5·9	
Lysine	5·5	5·2		4·5	
Methionine }	3·5†	1·2	2·8†	0·5	1·0†
Cystine }		1·6		0·5	
Threonine	4·0	3·2		2·5	
Valine	5·0	4·1		2·2	
Arginine	—	11·5		12·4	

* The part remaining after removal of gums and seed coat.
† Combined value for the two amino acids.

(3·40). Table 5 shows an inverse relationship between urinary N excretion (g/kg metabolic body size) ($W^{0.75}$) and BV. The lowest value of urinary N excretion (0·86 g/kg $W^{0.75}$) was recorded for whole defatted egg and the highest (2·69 g/kg $W^{0.75}$) for CGM protein isolate, corresponding to BV values of 0·93 and 0·36 respectively. The faecal N excretion remained at fairly constant levels (0·37–0·48 g/kg $W^{0.75}$) with the exception of SOM-45 (0·95 g/kg $W^{0.75}$) and CGM (1·17 g/kg $W^{0.75}$). This discrepancy might be explained on the grounds of product-purity since SOM-45 and CGM are not pure proteins and contain other material such as celluloses, hemicelluloses, pentosans, phenolic compounds and other organic substances. The excess N that appeared in the faeces with SOM-45 and CGM may have been due to an undigested portion of protein encapsulated in a fibrous coating within the plant tissue and so protected from the proteolytic enzymes of the gut (Drouliscos & Bowland 1969). It is also likely that in the CGM protein was precipitated due to the presence of the phenolic compounds.

The results recorded with feeding trials with rats show that at a level of inclusion of CGM in the diet of 250 g/kg the PER and NPU values were 1·66 and 0·58 compared with those recorded for dried tomato pomace (PER 2·18 NPU 0·55) (Drouliscos, 1976) and SOM-45 (PER 2·40 NPU 0·80) (Drouliscos & Bowland, 1969).

Supplementation with DL-methionine at 4 and 8 g/kg diet elicited a growth response but further addition (12 g/kg) had a depressing effect on growth, protein intake and BV (Table 6).

The addition of other essential amino acids improved growth, PER and BV, indicating a deficiency or an imbalance in the protein provided by the CGM (supplements 4 and 5) Table 6. Biological values were restored with supplements 4 and 5 (0·79 and 0·78) to the level recorded with supplement 1 (0·80) (Table 6). This response is indicative of a methionine deficiency since the inclusion of lysine in supplement 4 and of isoleucine, histidine and other essential amino acids in supplement 5 (Table 2) had no effect on the BV value. It is therefore likely that under the present experimental conditions methionine was the most limiting amino acid followed by leucine, isoleucine and threonine as second limiting. It could be of interest to see the response to leucine and isoleucine supplementation without methionine. This was not done in this study. Accordingly the value of PER of unsupplemented CGM was improved by 34·9% by supplement 4 and 56% by supplement 5 (Table 6). This suggests that the response in growth and PER was the result of the extra N intake (Table 6). It is not surprising that the lowest BV and NPU (by N balance) values

Table 4. *Comparison of the essential amino acid (AA) patterns (mg/g total essential AA) of carob* (Ceratonia siliqua) *germ meal (CGM)*, CGM protein isolate, Promine-D, soya-bean meal (SOM-45), casein (CS) and whole defatted egg*

	Protein source					
	CGM	CGM isolate	Promine-D	SOM-45	CS	Whole defatted egg
Phenylalanine	64	67	113	106	101	97
Isoleucine	76	62	91	76	87	77
Leucine	141	168	163	170	173	162
Lysine	126	129	146	143	157	125
Methionine–cystine	68	27	53	35	71	98
Valine	99	62	81	103	110	109
Threonine	77	72	73	97	82	89
Tyrosine	68	57	73	76	113	87
Arginine	282	356	162	140	59	116
Histidine	54	63	45	53	47	42
Total essential amino acid (mg/g nitrogen)	2564	2177	2680	2796	3283	3406
Essential amino acid content : N content	2·56	2·18	2·68	2·80	3·28	3·40
Essential amino acid index†	69·6	52·4	74·7	78·0	88·6	—

* The part remaining after removal of gums and seed coat.
† Calculated as described by Oser (1951).

Table 5. *Values for the utilizable nitrogen, biological value, urine and faecal N excretion (g/kg $W^{0.75}$) of rats in the experimental diets containing carob* (Ceratonia siliqua) *germ meal (CGM), CGM protein isolate, Promine-D, soya-bean meal (SOM-45), egg and casein (CS)*

(Values are means with their standard errors)

Diet...	Egg		CS		SOM-45		Promine-D		CGM		CGM protein isolate	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Mean body-wt (g)	92·46	—	81·04	—	72·75	—	73·40	—	79·76	—	67·97	—
Utilizable nitrogen*	11·20	0·110	11·81	0·157	4·71	0·130	7·28	0·101	4·11	0·094	5·01	0·210
Biological value	0·93	0·010	0·79	0·010	0·67	0·020	0·49	0·004	0·67	0·015	0·36	0·016
Urine N (g/kg $W^{0.75}$)	0·86	0·041	1·69	0·085	2·25	0·083	2·33	0·023	2·16	0·083	2·69	0·134
Faecal N (g/kg $W^{0.75}$)	0·48	0·035	0·38	0·016	0·95	0·044	0·40	0·051	1·17	0·019	0·37	0·026

$W^{0.75}$, metabolic body size.
* Percentage nitrogen in dry matter × net protein utilization.

Table 6. *Nutritional and biological indices for rats given experimental diets containing whole defatted egg, carob* (Ceratonia siliqua) *germ meal (CGM) and CGM protein isolate as protein sources*

(Mean values with their standard errors for six to ten rats for the PER, five rats for the BV and eight rats for the NPU)

Protein source	PER test						N-balance test							
	Body-wt gain (g)		Crude protein (nitrogen × 6·25) intake (g) 28 d		PER		BV		TD		NPU (BV × TD)		NPU by carcass analysis)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Egg	92·80	7·68	28·73	1·12	3·24	0·29	0·93	0·011	0·99	0·007	0·92	0·007	—	—
CGM: unsupplemented	40·30	3·38	24·20	1·92	1·66	0·09	0·67	0·015	0·87	0·004	0·58	0·013	0·59	0·051
+Supplement no. 1*	47·93	2·17	24·45	0·86	1·95	0·11	0·80	0·003	0·87	0·015	0·69	0·013	0·60	0·070
2	49·38	2·60	24·51	2·15	2·01	0·11	0·78	0·015	0·85	0·023	0·66	0·026	0·63	0·007
3	41·30	1·98	21·71	0·84	1·90	0·11	0·74	0·011	0·86	0·022	0·63	0·020	0·56	0·001
4	61·70	4·10	27·35	0·93	2·24	0·08	0·79	0·021	0·93	0·011	0·73	0·015	0·77	—
5	90·40	8·51	34·55	1·26	2·59	0·20	0·78	0·012	0·92	0·021	0·71	0·019	0·71	0·045
CGM protein isolate:	—		—		—		0·36	0·016	0·97	0·003	0·35	0·015	—	
+Supplement no. 6	—		—		—		0·66	0·013	0·97	0·004	0·64	0·013	—	

BV biological value; PER protein efficiency ratio; TD true digestibility; NPU net protein utilization.
* For details, see Table 2.

were recorded for CGM protein isolate (BV 0·36, NPU 0·35) (Table 6) since as shown in Table 1 the methionine and cystine content is one-third of the value recorded for the CGM. Supplementation of CGM protein isolate with methionine and other amino acids (supplement 6; Table 2) raised the BV value to 0·66. Due to the limited quantity of the isolate that was available for the bioassays the PER was not carried out. The poor performance of CGM protein isolate as judged by the low NPU (0·35) is in close agreement with values reported by Shemer & Perkins (1974) for soya-bean protein isolate (Promine-D), the range was 0·24–0·36.

It is interesting to note that in the present study the BV obtained with Promine-D was 0·49 (Table 5) with value for NPU (BV × TD) of 0·47. This value is higher than the value of 0·29 reported by Shemer & Perkins (1974) for Promine-D. The recorded value could have been due to the heavier and older rats used for the NPU determination resulting in lower N retention.

On the basis of the previously mentioned observations some conclusions may be drawn regarding the protein quality of CGM and its isolate. With CGM used in the present study at 250 g/kg diet to supply all the protein, growth in rats was 43·4% of that with the egg diet. The addition of amino acids (supplements nos. 1, 2, 4 and 5, Table 2) progressively improved body-weight gain to a maximum of 97·4% (Table 6). Supplement no. 3 (12 g DL-methionine/kg) reduced growth and protein intake (Table 6) suggesting that the methionine added was in excess and could have created an amino acid imbalance or a slight toxicity. Similar observations have been reported by Drouliscos (1976) with dry tomato pomace supplemented with 5 g methionine/kg diet in rats, by Muramatsu *et al.* (1971) with the addition of 50 g methionine/kg diet in rats and by Smith *et al.* (1975) with 2 g methionine/kg diet (*Aspergillus oryzae* biomass) in pigs.

CGM could replace part of the protein portion of a compounded feed for single-stomached species provided its amino acid deficiencies are corrected. Experiments with broiler chicks are in progress and the observations made will be reported in a later communication.

There appears to be some indication that during the preparation of the protein isolate under the experimental conditions of this study a certain loss of the sulphur containing amino acids is taking place together with some other essential amino acids such as lysine, valine, threonine and isoleucine (Table 1).

It should therefore be pointed out that although isolates appear to have a high CP content, they may not necessarily be well balanced in terms of their amino acid composition.

It is in this context that the incorporation of such products in various food preparations should be carefully planned and the required amino acids added if necessary.

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