COMMUNICATIONS

Nutritional Evaluation by Rat Feeding of Preparations from Guar Seed (*Cyamopsis tetragonoloba*) Including Supplementation with Lysine and Methionine

Guar (*Cyamopsis tetragonoloba*) meal is the product left after extraction of the gum from the seed by the guar gum industry. The meal was treated with hot water and 1*N* HCl to remove growth-inhibitory principles. The guar meal samples at a 10% protein level with or without DL-methionine and L-lysine monohydrochloride supplements were tested with rats for growth rate, protein efficiency ratio (PER), and effect on organs. Feeding raw guar meal alone or with amino acids caused a high degree of mortality in the experimental animals. Hot water treatment improved the utilization of the guar meal, and acid treatment further improved its utilization, as shown by PER values and nitrogen content of organs, plasma, and hemoglobin of blood. Amino acid supplement of the two differently treated guar meals further enhanced growth and PER values. The per cent lipid content of the liver, which was high in the groups fed treated or untreated guar meal, was reduced when the acid-treated guar meal was supplemented with amino acids.

Guar (*Cyamopsis tetragonoloba*) is a drought-resistant summer legume crop, grown principally for gum production. The bean remaining after extraction of the gum contains about 36 to 45% protein, which provides adequate amounts of all amino acids for optimum rat growth, except lysine and methionine (Van Etten *et al.*, 1961). Attempts to feed the raw guar protein to animals have met with varying degrees of success. Sathe and Bose (1962) and Ogra *et al.* (1963) reported that a 12.5% level of guar meal in the rations of starter chicks resulted in poor growth performance and feed efficiency. Borchers and Ackerson (1950), Arrington *et al.* (1955), and Kawatra and Sidhu (1968) reported a low growth rate of rats fed diets with guar meal as the protein source. Preliminary findings in this laboratory show that when raw guar meal is extracted with hot water or with 1*N* HCl, utilization is much improved, acid treatment giving the best results. The growth performance of rats fed the acid-treated guar meal was, however, lower than that of a casein-fed group. This may be attributed to the known deficiency of lysine and methionine. The present investigation was undertaken to study with growing rats the effect of different treatments of the raw guar meal and the addition of lysine and methionine to the meal.

EXPERIMENTAL

Guar meal is the by-product of extraction of gum from guar seed by the guar gum industry. The meal was procured from Hindustan Gum and Chemicals, Ltd., Bhiwani.

Acid Treatment. Seventy grams of guar meal sieved through a 1-mm. sieve was added to 400 ml. of 1*N* HCl in a beaker and kept in a boiling water bath for one-half hour. It was then filtered and washed with water (3 liters) until the residue was free of acid. The residue was pressed and dried overnight in a hot air oven at 80° C. The yield of the product was 60%.

Hot Water Treatment. The guar meal (100 grams) was sieved through a 1-mm. sieve, added to about 1 liter of water, and boiled for an hour. The meal was filtered through cloth, washed five times with 500 ml. of water, pressed, and dried as before. The yield was 77%.

The treated samples along with raw guar meal were analyzed for nitrogen by the micro-Kjeldahl method (McKenzie and Wallace, 1954); ether extract, crude fiber, ash, and moisture were determined by the procedure of the Association of Official Agricultural Chemists (1960). The samples were also analyzed for total lysine and methionine according to the method of Moore *et al.* (1958) and Horn *et al.* (1946), respectively. The chemical composition of treated and untreated samples is given in Table I.

An experiment was designed to study the effect of different treatments and of supplementation by DL-methionine and L-lysine monohydrochloride on the rate of growth, protein efficiency ratio (PER), and effect on different organs of albino rats, using casein as the reference diet. Three experimental rations (D_2, D_3, and D_4), incorporating raw, hot water-treated, and acid-treated guar meal, respectively, were compared to a reference diet, D_1, containing casein in place of guar meal. Diets D_5, D_6, and D_7 contained DL-methionine and L-lysine monohydrochloride supplements to raw, hot water-, and acid-treated guar meals, respectively. Casein contained methionine (3.22 grams per 16 grams of N) and lysine (8.50 grams per 16 grams of N) and the amino acids added were such that the total amount of the amino acids in the diets was equal to the quantity present in the reference diet. All substitutions were made on a 10% protein basis. The composition of experimental diets is given in Table II.

Table I. Chemical Composition of Guar Meal Samples

Guar Meal Sample	Moisture, %	Crude Protein (N × 6.25), %	Crude Fiber, %	Ether Extract, %	Ash, %	Methionine, G./16 G. N	Lysine, G./16 G. N
Raw	6.80	45.60	10.42	3.41	4.60	1.64	6.95
HCl-treated	6.20	46.37	12.50	11.60	3.65	1.76	6.77
Hot water–treated	7.00	49.90	11.70	7.70	4.10	1.49	6.77

Table II. Composition of Diets

Ingredients	D_1	D_2	D_3	D_4	D_5	D_6	D_7
Vitamin mixture[a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt mixture[b]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Dalda[c]	10.00	9.26	8.50	6.80	9.26	8.50	6.80
Cellulose	5.00	2.70	2.70	1.80	2.70	2.70	1.80
Casein	11.40	...	...	...	...	...	...
Raw guar meal	...	21.90	...	...	21.90	...	...
Hot water–treated guar meal	...	...	20.04	...	...	20.04	...
Acid-treated guar meal	...	...	...	21.55	...	...	21.55
Sucrose	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Starch	63.60	56.14	58.76	59.85	55.807	58.387	59.502
DL-Methionine	...	...	...	...	0.167	0.149	0.172
L-Lysine monohydrochloride	...	...	...	...	0.166	0.224	0.176
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[a] Vitamin mixture recommended by Chapman *et al.* (1959) for rats.
[b] Salt mixture described by Oser (1965).
[c] Hydrogenated fat manufactured by Hindustan Lever, Ltd., Bombay.

Table III. Body Weight Gain, Food Intake, PER, and Weight of Liver, Spleen, and Right Gastrocnemius Muscle of Rats Fed Treated Guar Diets for 4 Weeks

(Values are mean ± S.E. of mean)

Diet[a]	Wt. Gain, G.	Food Consumed, G.	PER[b]	Liver Wt., G.		Spleen Wt., G.		Kidney Wt., G.		Rt. Gastrocnemius Wt., G.	
				Total	Per 100 g. body wt.	Total	Per 100 g. body wt.	Total	Per 100 g. body wt.	Total	Per 100 g. body wt.
D_1	52.20	230.30	2.26	3.040	3.246	0.289	0.279	0.699	0.747	0.549	0.565
	±3.27	±9.43	±0.10	±0.142	±0.060	±0.030	±0.032	±0.032	±0.018	±0.036	±0.019
D_2	−14.00	95.10	−Ve	1.165	4.480	0.040	0.154	0.290	1.110	0.080	0.308
D_3	3.70	151.80	0.22	1.990	4.371	0.127	0.281	0.459	1.050	0.234	0.510
	±0.69	±8.51	±0.05	±0.080	±0.190	±0.011	±0.032	±0.088	±0.043	±0.015	±0.022
D_4	14.50	199.80	0.72	2.210	3.957	0.145	0.227	0.530	0.950	0.305	0.548
	±1.80	±7.25	±0.28	±0.110	±0.189	±0.017	±0.022	±0.030	±0.030	±0.017	±0.015
D_6	13.70	136.60	1.00	2.450	4.459	0.146	0.249	0.512	0.933	0.301	0.560
	±1.74	±6.67	±0.12	±0.078	±0.161	±0.018	±0.033	±0.018	±0.028	±0.017	±0.028
D_7	44.70	235.10	1.89	3.710	4.420	0.240	0.272	0.711	0.815	0.564	0.648
	±2.71	±5.90	±0.08	±0.190	±0.109	±0.023	±0.023	±0.018	±0.024	±0.065	±0.101

[a] As described in Table II.
[b] Protein efficiency ratio.

Pure-bred albino rats, about 4 weeks old, were individually weighed and randomly divided into seven groups, each consisting of seven rats. The animals were housed individually in cages having wire-mesh bottoms, and had free access to water. The diets were fed *ad libitum* for 4 weeks.

Daily food intake of individual rats was recorded during the experimental period. The animals were weighed at weekly intervals. At the end of the experimental period the animals were anesthesized with solvent ether and blood was collected from the aorta in oxalated tubes. The liver, spleen, kidneys, and right gastrocnemius muscle were weighed after removing the connective tissues. The liver was analyzed for total nitrogen by a micro-Kjeldahl method, and for glycogen by the method of Seifter *et al.* (1950). The fat content was determined by a Soxhlet extraction method (AOAC, 1960) after the liver was triturated with anhydrous sodium sulfate. The hemoglobin content of the blood was determined by the acid hematin method (Wintrobe, 1958). The blood was centrifuged at 2500 r.p.m. for 15 minutes, and the plasma was separated and analyzed for total nitrogen.

RESULTS AND DISCUSSION

The mean weight gain of experimental rats in the different groups, the quantity of food consumed, the PER of the test, and the diets are given in Table III.

Acid-treated guar meal with added methionine and lysine produced the best results. The weight gain in this group approached that of the casein-fed reference diet group. In the raw guar meal–fed group the animals lost weight and their food intake was lowest. The weight gain, food consumption, and PER value in animals fed the D_7 diet were appreciably higher than those with raw or hot water–treated guar meal, with or without amino acid supplement. Even hot water treatment produced considerable improvement compared with raw guar meal. While amino acid supplement to the raw guar meal had no effect, the same supplement to the hot water–treated guar meal was beneficial. This suggests that acid treatment of guar meal gives a product which the rat can utilize; the product obtained by hot water treatment, though better than that from untreated meal, still contains growth-inhibitory substances. The acid-treated sample of guar meal, when supplemented with the deficient amino acids, gave growth and PER values near those of the casein-fed control group. The mortality of rats in groups D_2 and D_5 was 86 and 100%, respectively, confirming the presence of growth-inhibitory and toxic factors in the untreated guar meal. Post-mortem examination of rats showed that the liver and other organs looked normal, except that the intestine was filled with gases and a dark fluid which appeared hemorrhagic.

Total liver weights of animals fed the experimental diets were appreciably smaller than those of the control group (Table III); in the D_7-fed group it was slightly greater than in the control group. When the weights were expressed per 100 grams of body weight, the D_2 group showed larger livers than the control group. The total spleen and right gastro-

Table IV. Blood Hemoglobin and Total N of Plasma and Liver, and Lipid and Glycogen Contents of Liver of Rats Fed Treated Guar Meal Diets for 4 Weeks

(Values are mean ± S.E. of mean)[a]

	Liver			Blood	
Diet[b]	Total N, mg./g.	Glycogen, mg/g.	Total lipids, mg./g.	Hemoglobin content, g./100 ml.	Plasma total N, mg./100 ml.
D_1	31.85	42.90	30.40	14.70	677.00
	±0.40	±7.07	±3.11	±0.13	±47.17
D_2	25.90	3.90	51.80	8.80	532.00
D_3	26.02[c]	84.60	58.20[c]	13.80	554.40
	±0.69	±11.45	±2.89	±0.54	±54.24
D_4	25.63[c]	73.70	52.70[c]	14.20	586.20
	±0.77	±7.64	±3.82	±0.61	±61.11
D_6	25.78[c]	64.40	52.70[d]	13.20	572.60
	±0.49	±5.90	±8.14	±0.25	±38.18
D_7	29.88[d]	83.60	21.20	14.30	695.80
	±0.75	±6.49	±5.35	±0.53	±61.76

[a] Statistical analysis by Student's t test comparing with control group $P > 0.05$ not significant.
[b] As described in Table II.
[c] $P < 0.01$.
[d] $P < 0.05$.

Table V. Total N of Spleen, Muscle, and Kidneys of Rats Fed Treated Guar Meal for 4 Weeks

(Values are mean ± S.E. of mean)

	Spleen Total N, Mg.		Right Gastrocnemius Muscle Total N, Mg.		Kidney Total N, Mg.	
Diet[a]	Per g.	Per total spleen	Per g.	Per total muscle	Per g.	Per total kidney
D_1	31.88	9.01	34.97	19.05	23.02	15.48
	±1.43	±0.65	±0.99	±1.56	±0.37	±0.47
D_2	28.00	1.12	41.12	3.29	24.37	7.07
D_3	29.92	3.82	34.24	8.04	26.45	12.04
	±1.40	±0.43	±1.46	±0.68	±1.35	±0.47
D_4	29.03	3.75	32.68	9.98	25.03	13.13
	±0.86	±0.49	±0.72	±0.58	±1.22	±0.37
D_6	30.96	4.58	33.91	10.17	26.39	13.36
	±0.76	±0.56	±1.25	±0.56	±0.98	±0.80
D_7	28.26	6.76	31.38	16.71	23.14	16.31
	±1.53	±0.81	±2.67	±0.91	±0.71	±0.58

[a] As described in Table II.

cnemius muscle weights in the animals were appreciably smaller in the rats fed the raw and treated guar meal diet than in the casein-fed animals. Sidransky (1960) observed that animals fed plant proteins deficient in essential amino acids lose body weight, accompanied by differential weight loss in various organs. The organ weights in the group fed acid-treated guar meal supplemented with amino acids were nearly the same as in the group fed the D_1 control diet.

The data on total nitrogen, glycogen, and lipids of the liver are given in Table IV. The total nitrogen, expressed as milligrams per gram of liver, was significantly lower in all the guar meal–fed groups compared to the D_1-fed group, but the nitrogen content of the D_7 group was closer to that in the control group. The amino acid supplement to the hot water–treated guar meal did not improve the liver nitrogen values. Improved growth in rats and increase in liver nitrogen were obtained by supplementing the rice-legume diet with the deficient amino acids (Wagle *et al.* 1962). The glycogen content per gram of liver in the differently treated guar meal–fed groups was greater than in the raw guar meal–fed group. However, the glycogen content was appreciably higher in the D_7-fed group than in the control D_1 group. Analysis showed that the animals fed the treated or untreated guar meal (D_2, D_3, D_4, and D_6 diets) had higher liver lipid contents than the casein-fed group. While addition of amino acids to the hot water–treated guar meal did not change the lipid level, the same supplement to the acid–treated guar meal considerably reduced the lipid concentration. There was a considerable fall in the hemoglobin content and plasma nitrogen of rats fed the raw guar meal diet (D_2) compared to those on the casein diet (Table IV). The values of these parameters were improved in the D_7-fed group and were close to those of the control group. This is corroborated by the data on nitrogen content of the different organs (Table V). The total nitrogen of spleen, muscle, and kidneys expressed as milligrams per gram showed no significant difference in the different groups, but when the data were expressed on per total organ basis, the nitrogen content in different guar meal–fed groups was low compared to the casein-fed group (D_1). However, the nitrogen content of these organs in the D_7 group was appreciably higher than in other guar meal–fed groups and close to the D_1-fed group. This may be anticipated; with the amino acid supplement, all the amino acids are now available at the site of protein synthesis and thus may increase the nitrogen content of the different organs.

Acid and hot water treatment of the meal improved the utilization of guar protein; however, the PER values in the animals fed these diets were low compared to those in the casein diet–fed animals. The improvement in growth resulting from supplementation of the treated guar meal diet with the deficient amino acids may be expected. Similar nutritional improvements have been observed with rice diets (Harper *et al.*, 1955; Pecora, 1953). The beneficial effect of amino acid supplement to the acid-treated guar meal suggests that this effect can be observed after the meal has been detoxified.

ACKNOWLEDGMENT

The authors express their thanks to I. S. Bhatia, Department of Chemistry and Biochemistry, for his interest in this study. Thanks are also due to Hindustan Gum and Chemicals, Ltd., Bhiwani (Haryana), for providing guar meal samples used in this investigation.

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Received for review October 25, 1968. Accepted February 18, 1969