

Use of a Four-Parameter Logistic Model to Evaluate the Protein Quality of Mixtures of Mormon Cricket Meal and Corn Gluten Meal in Rats¹

MARK D. FINKE, GENE R. DEFOLIART AND NORLIN J. BENEVENGA

Departments of Nutritional Science, Entomology and Meat and Animal Science, University of Wisconsin-Madison, Madison, WI 53706

ABSTRACT Weanling Sprague-Dawley rats were fed diets containing corn gluten meal (CGM), Mormon cricket meal (MCM), MCM supplemented with methionine (MCM + Met) or CGM-MCM mixtures as the sole source of dietary protein in purified diets. Animal response (weight or nitrogen gain) was analyzed as a function of nitrogen intake and described by a series of curves using a four-parameter logistic model. Parameter sharing was used to differentiate statistically between the response curves. When used for maximum nitrogen retention the quality of the protein sources could be ranked as follows: MCM + Met > 40 CGM-60 MCM > 50 CGM-50 MCM > 60 CGM-40 MCM > MCM > CGM. When used for maximum weight gain the ranking was as follows: MCM + Met > 40 CGM-60 MCM > 50 CGM-50 MCM = 60 CGM-40 MCM > MCM > CGM. The rankings of the protein sources when used for weight maintenance or nitrogen equilibrium were similar to those seen for maximum weight or nitrogen gain except for the ranking of MCM, which changed from fifth to first. These results show MCM to be a good quality protein source and that methionine is the first limiting amino acid when used for growth but not for maintenance. The shape of the response curves was dependent upon the response criteria as well as the source of dietary protein. The factors that may affect the shape of the dose-response curves and the application of nonlinear models toward animal feeding programs are discussed. *J. Nutr.* 117: 1740-1750, 1987.

INDEXING KEY WORDS:

- rat • insect protein • corn gluten meal
- protein mixtures • protein quality
- nonlinear models • logistic model
- parameter sharing

In the 1960s investigations into the nutritional potential of housefly pupae as a source of protein for use in poultry diets were initiated. Dried housefly pupae were found to be an adequate protein substitute for soybean meal and were a good source of vitamins and

trace minerals (1-3). Unfortunately no attempt was made to determine which amino acids were limiting. In the Far East an extensive sericulture industry produces silkworm pupae as a by-product and these have been utilized by the poultry industry as a substitute for fish meal (4, 5).

Limited information is available on the quality of insect protein. Quantitative studies designed to evaluate the protein quality of the termite species, *Macrotermes falciger*, used the protein efficiency ratio method (6). When rats were fed termite protein it was found to have a value of 1.7, compared to values of 2.4, 1.7 and 1.5 for casein, peanut meal and wheat gluten, respectively. The low quality of termite protein was attributed to its low digestibility. The termite larvae were fried in order to simulate the preparation used by native Africans. The high frying temperature may have altered the quality of the protein. Because poultry consume insects as part of their natural diet, Phelps, Struthers and Moyo (6) suggested that they may utilize insects more efficiently than other animals.

With these results in mind we initiated an investigation to evaluate the protein quality of the Mormon cricket, *Anabrus simplex* Haldeman. Mormon crickets were used as a representative of the class Orthoptera, a group that includes grasshoppers, crickets and locusts, and because their banding behavior allows for the collection of the large quantities needed for nutritional experiments. Of note is that the Mormon cricket is used as a food source by a number of different species and at one time was a major food of certain North American Indians (7, 8).

Results from experiments using poultry indicated that ground Mormon crickets were an adequate substitute

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for soybean meal in practical poultry rations (9, 10). In addition, it was found that methionine and arginine were colimiting when ground Mormon crickets were the sole source of dietary protein in purified diets. The relatively high arginine requirement of poultry and the low levels of arginine found in ground Mormon crickets suggest that poultry may not utilize insect protein as efficiently as other animals. Therefore, a series of experiments was designed to evaluate the quality of Mormon cricket protein when fed to rats. The quality of Mormon cricket protein was evaluated alone, when mixed with a complementary protein source and when supplemented with methionine. Amino acid analysis (9) indicated that the methionine plus cystine concentration of Mormon cricket meal (MCM) was 47% of that of soybean meal suggesting that methionine would be first limiting. This was later confirmed in preliminary feeding trials. Corn gluten meal (CGM) was selected as a complementary protein source because of its high methionine content (11).

A four-parameter logistic model was used to describe the dose-response relationship and protein quality was evaluated by comparing the nitrogen intake required to achieve identical levels of performance (12).

MATERIALS AND METHODS

Diets. All diets contained a vitamin mix, 0.5% (13); a mineral mix, 5.0% (13) (Teklad Test Diets, Madison, WI); corn oil, 5.0% (Archer Daniels Midland, Decatur, IL); choline chloride, 0.2% (U.S. Biochemical, Cleveland, OH); cellulose, 0.2% (U.S. Biochemical) (used as a carrier for the choline chloride); and equal weights of cornstarch (Staley, Decatur, IL) and glucose monohydrate [cerelose, Corn Products (CPC) International, Englewood Cliffs, NJ]. The two protein sources, MCM (crude protein content, 67.4%) and CGM (crude protein content, 58.3%, Teklad Test Diets), were added to the diet at the expense of equal weights of cornstarch and glucose monohydrate. Although the mineral mix used has been shown to contain an inadequate level of zinc when used in amino acid diets, the possibility of zinc limiting animal growth is unlikely in this experiment as both MCM and CGM contained adequate levels of zinc (118 and 19 mg/kg, respectively).

Six protein sources were evaluated in this experiment. Three CGM-MCM protein mixtures were tested: 60 CGM-40 MCM, 50 CGM-50 MCM and 40 CGM-60 MCM. The numerical designation of the protein mixtures stands for the percentage of the total dietary protein supplied by each of the two protein sources so that for the 60 CGM-40 MCM series, 60% of the dietary protein was supplied by CGM and 40% by MCM. In addition, CGM and MCM were also evaluated separately. The other protein source tested was MCM supplemented with 0.4% of L-methionine (U.S. Biochemical). For each protein source tested, 12 dietary levels

were used. The dietary protein concentrations selected were not the same for all protein sources tested (Table 1) and were based on the protein value calculated from the amino acid composition. These levels were selected in an attempt to provide diets that would show a full range of animal responses, from maximum weight loss to maximum weight gain. A diet containing no added protein was formulated to serve as a baseline control. The diets were fed in powdered form in metal feed cups. Diet and water were available ad libitum.

Adult Mormon crickets (*A. simplex* Haldeman) were collected in Moffat County, CO, and frozen immediately after collection. The crickets were kept frozen until needed, at which time they were dried by lyophilization and then ground in a Wiley mill. The lipid material was removed by refluxing with diethyl ether for 24 h and the dry powder was redried at 45°C for 6 h to remove any residual ether. Both protein sources were passed through a 40 mesh screen before use.

Animals. Twenty-one-day-old weanling male Sprague-Dawley rats (Sprague-Dawley Farms, Madison, WI) were divided into 76 groups so that the mean weights of the groups were similar (average 54 g; range 47-60 g). Groups of four rats were assigned to each of the 73 dietary treatments. In addition, three groups were killed at the beginning of the experiment to obtain an estimate of the initial body nitrogen. Rats were individually housed in stainless steel wire-bottom cages and maintained at 25°C on a 24-h photoperiod (12 h light, 12 h dark). The length of the experiment was 21 d; rats were weighed daily and food intake was determined three times per week. At the end of the experiment the rats were killed by ether inhalation and the gastrointestinal tract of each rat was removed, washed free of its contents and returned to the carcass. The carcasses were placed in individual airtight plastic bags and frozen until needed for nitrogen analysis.

To prepare each rat carcass for nitrogen analysis it was chopped into 5-10 small pieces, lyophilized and refrozen. Just before grinding, the pieces were immersed in 500 ml of liquid nitrogen for 5 min and then ground in a Wiley mill at -20°C. The resulting powder was used for nitrogen analysis. A micro-Kjeldahl procedure with ammonia determination via a phenol-hypochlorite reaction was used to determine carcass and dietary nitrogen content (14). All nitrogen analyses were done in triplicate.

The nitrogen content of the 12 rats killed at the beginning of the experiment was plotted as a function of body weight and a regression equation determined. The initial body nitrogen content of the rats fed the experimental diets was then estimated from the following regression equation:

$$Y = 0.0594 + 0.0256(X),$$

where Y = initial body nitrogen and X = initial body weight.

The data from this experiment were analyzed with

TABLE 1

Dietary nitrogen content, food intake, weight gains and nitrogen gain of rats fed diets containing corn gluten meal (CGM) or Mormon cricket meal (MCM) alone or in mixtures as a source of dietary protein¹

Protein source	Dietary nitrogen content	Food intake	Weight gain	Nitrogen gain	
100 CGM	8.40	212.0 ± 3.3	90.8 ± 3.1	2.56 ± 0.09	
	8.15	213.3 ± 2.3	94.3 ± 1.4	2.69 ± 0.06	
	7.17	206.0 ± 4.1	78.5 ± 2.3	2.29 ± 0.00	
	6.32	223.0 ± 12.3	81.3 ± 6.3	2.08 ± 0.17	
	5.61	202.3 ± 3.4	62.8 ± 1.4	1.62 ± 0.08	
	4.89	205.0 ± 4.4	55.8 ± 2.1	1.43 ± 0.04	
	4.01	176.5 ± 13.2	41.5 ± 5.3	1.17 ± 0.10	
	3.37	179.3 ± 1.9	31.5 ± 2.8	0.93 ± 0.04	
	2.51	151.3 ± 2.0	14.8 ± 0.9	0.52 ± 0.04	
	1.70	125.5 ± 2.6	3.0 ± 1.2	0.13 ± 0.02	
	0.87	125.8 ± 1.8	-0.8 ± 1.0	0.04 ± 0.01	
	0.43	115.0 ± 0.9	-4.8 ± 2.0	-0.08 ± 0.02	
	60 CGM 40 MCM	4.77	295.8 ± 10.3	112.5 ± 7.1	3.63 ± 0.24
		4.00	322.0 ± 6.3	125.3 ± 5.2	3.96 ± 0.15
		3.23	321.3 ± 3.2	106.5 ± 3.4	3.18 ± 0.06
		2.91	290.0 ± 7.3	91.0 ± 3.5	2.57 ± 0.09
2.57		308.5 ± 11.3	88.8 ± 5.6	2.38 ± 0.13	
2.31		295.5 ± 10.3	78.0 ± 8.5	1.96 ± 0.15	
1.98		243.3 ± 16.2	48.5 ± 4.6	1.28 ± 0.09	
1.60		236.5 ± 11.3	39.0 ± 3.1	0.94 ± 0.03	
1.36		200.0 ± 5.3	28.3 ± 1.0	0.65 ± 0.01	
1.05		197.3 ± 7.9	17.7 ± 1.7	0.36 ± 0.05	
0.70		167.8 ± 14.5	6.5 ± 1.7	0.13 ± 0.04	
0.36		117.0 ± 8.0	-6.8 ± 1.9	-0.14 ± 0.04	
50 CGM 50 MCM	5.01	302.3 ± 6.5	118.3 ± 2.0	3.83 ± 0.08	
	4.13	300.8 ± 9.3	118.8 ± 4.5	3.75 ± 0.14	
	3.44	339.3 ± 24.8	115.8 ± 12.8	3.48 ± 0.36	
	3.03	327.3 ± 9.1	107.3 ± 6.0	3.15 ± 0.13	
	2.70	328.0 ± 16.1	96.3 ± 3.9	2.67 ± 0.09	
	2.34	313.5 ± 5.1	89.0 ± 3.8	2.47 ± 0.05	
	2.08	261.8 ± 6.9	53.3 ± 4.3	1.56 ± 0.04	
	1.75	266.0 ± 7.7	59.5 ± 1.3	1.31 ± 0.08	
	1.37	230.3 ± 10.9	36.3 ± 3.6	0.87 ± 0.04	
	1.02	212.7 ± 19.2	22.0 ± 2.2	0.58 ± 0.01	
	0.77	153.3 ± 5.9	6.5 ± 0.9	0.14 ± 0.01	
	0.32	120.0 ± 2.7	-6.8 ± 1.0	-0.14 ± 0.02	
40 CGM 60 MCM	4.84	308.5 ± 4.7	122.5 ± 3.2	3.89 ± 0.12	
	4.09	296.8 ± 4.2	116.3 ± 3.6	3.59 ± 0.07	
	3.25	320.5 ± 24.4	119.5 ± 12.7	3.59 ± 0.36	
	2.94	327.3 ± 11.0	106.5 ± 3.7	3.16 ± 0.03	
	2.53	313.8 ± 13.4	106.8 ± 5.8	3.08 ± 0.20	
	2.28	310.5 ± 6.5	90.3 ± 6.9	2.43 ± 0.16	
	1.97	305.0 ± 7.8	84.0 ± 6.1	2.32 ± 0.11	
	1.65	258.8 ± 10.1	55.3 ± 5.8	1.59 ± 0.10	
	1.33	243.0 ± 7.7	44.8 ± 2.2	1.09 ± 0.05	
	0.99	210.0 ± 13.4	27.3 ± 3.2	0.68 ± 0.02	
	0.66	161.0 ± 1.8	9.3 ± 0.9	0.26 ± 0.02	
	0.35	119.3 ± 12.6	-5.8 ± 1.2	-0.09 ± 0.03	
MCM	6.41	288.3 ± 6.3	112.0 ± 6.2	3.80 ± 0.24	
	5.62	280.5 ± 9.0	118.8 ± 3.5	4.13 ± 0.04	
	4.74	283.3 ± 3.5	105.5 ± 3.2	3.49 ± 0.06	
	4.32	287.0 ± 12.8	104.5 ± 4.4	3.49 ± 0.17	
	3.87	280.5 ± 13.4	103.0 ± 7.3	3.27 ± 0.18	
	3.39	290.8 ± 7.1	99.3 ± 4.5	3.17 ± 0.08	
	2.98	273.8 ± 7.6	88.5 ± 6.3	2.75 ± 0.18	
	2.42	275.5 ± 12.2	78.3 ± 4.5	2.32 ± 0.10	
	1.90	239.0 ± 12.6	59.8 ± 3.7	1.69 ± 0.05	
	1.45	213.0 ± 8.6	37.8 ± 2.0	0.97 ± 0.06	
	0.96	175.8 ± 9.7	20.5 ± 2.1	0.52 ± 0.06	
	0.47	130.0 ± 1.9	-3.0 ± 2.4	-0.07 ± 0.04	

TABLE 1 *Continued*

Protein source	Dietary nitrogen content	Food intake	Weight gain ¹	Nitrogen gain
MCM plus methionine	3.28	298.0 ± 7.9	112.3 ± 3.9	3.67 ± 0.15
	2.98	292.5 ± 16.3	114.0 ± 6.6	3.67 ± 0.24
	2.63	297.5 ± 7.5	113.3 ± 1.6	3.58 ± 0.04
	2.33	300.5 ± 7.8	106.3 ± 3.5	3.19 ± 0.07
	2.05	314.5 ± 11.6	104.0 ± 5.8	3.02 ± 0.14
	1.87	298.8 ± 7.6	91.0 ± 0.9	2.60 ± 0.06
	1.70	295.0 ± 3.0	83.3 ± 2.4	2.52 ± 0.06
	1.53	295.0 ± 9.9	74.5 ± 1.2	2.13 ± 0.05
	1.36	265.5 ± 8.3	60.0 ± 4.2	1.73 ± 0.07
	1.06	239.0 ± 7.9	39.3 ± 2.2	1.06 ± 0.07
	0.71	165.5 ± 6.5	12.5 ± 2.6	0.32 ± 0.06
	0.39	119.3 ± 5.2	-6.3 ± 1.0	-0.12 ± 0.03
	Protein free	0.14	97.0 ± 5.0	-14.3 ± 0.9

¹All values represent the mean ± SEM of four values, except for the rats fed the 0.99% nitrogen diet containing 60 CGM-40 MCM in which one rat was removed due to an unexplained weight loss.

a four-parameter logistic equation (12, 15). The model and a description of the parameters used are shown below.

$$\text{Response} = \frac{R_{\max} + [b(1+c) - R_{\max}]d^I}{1 + cd^I}$$

where b is the predicted response of rats fed a protein free diet, c is the shaping parameter, d is the scaling parameter, R_{\max} is the predicted maximum response and I = nitrogen intake.

All dose-response curves (weight or nitrogen gain versus nitrogen intake) were fitted with a simultaneous curve-fitting routine previously described (12, 16). Although the values for individual rats were used in the curve-fitting routines, only the mean values for each treatment are shown in the figures. For ease of interpretation the data were analyzed as two separate experiments, the first as a protein complementation experiment to evaluate the protein quality of various CGM-MCM mixtures and the second as an amino acid supplementation experiment to evaluate MCM with and without methionine supplementation.

RESULTS

The food intake, body weight gain and body nitrogen gain of each group of rats is shown in Table 1. When CGM was the sole dietary protein source no rat reached a peak value for body weight gain (120–125 g) or body nitrogen gain (3.7–4.1 g) despite dietary nitrogen concentrations in excess of 8%. In contrast, a plateau for both body weight and nitrogen gain could be obtained at substantially lower nitrogen concentrations with other protein sources. Generally, food intake peaked at a dietary nitrogen level below that needed to maximize body weight or nitrogen gain. This indicates that rats overeat when dietary nitrogen concentration intake approaches the requirement.

The final parameter estimates for the fit of the model to the data when weight gain was the dependent variable in the CGM-MCM protein complementation study are shown in Table 2. As expected (12), when all curves were constrained to share a common value for parameters b and R_{\max} no significant effect on the fit of the model to the data was noted. A common value for pa-

TABLE 2

Final parameter estimates of the weight gain–nitrogen intake response curves for rats fed diets containing corn gluten meal (CGM), Mormon cricket meal (MCM) or CGM–MCM mixtures as a source of dietary protein

Protein source	Parameter ¹			
	b	c	d	R_{\max}
CGM	-12.554 ± 1.001	1.022 ± 0.407	0.898 ± 0.011	129.986 ± 2.565
60 CGM-40 MCM	-12.554 ± 1.001	1.646 ± 0.360	0.774 ± 0.017	129.986 ± 2.565
50 CGM-50 MCM	-12.554 ± 1.001	1.646 ± 0.360	0.774 ± 0.017	129.986 ± 2.565
40 CGM-60 MCM	-12.554 ± 1.001	1.646 ± 0.360	0.738 ± 0.017	129.986 ± 2.565
MCM	-12.554 ± 1.001	-0.152 ± 0.182	0.875 ± 0.019	129.986 ± 2.565

¹ b = estimated weight gain of rats fed zero protein for 21 d; R_{\max} = estimated maximum 21-d weight gain; mean ± SD.

parameter c (shape) could be shared by the curves describing the weight gain of rats fed diets containing the three CGM-MCM protein mixtures. Identical dose-response curves sharing values for b , c , d and R_{\max} described the response of rats fed diets containing the 60 CGM-40 MCM and 50 CGM-50 MCM protein mixtures.

The five dose-response curves fitted to the weight gain-nitrogen intake data are shown in Fig. 1. The curve describing the response of rats fed diets that contained CGM as the sole source of protein had the smallest slope. The curves describing the response of rats fed diets containing the other four protein sources were similar up to weight gains of 60 g and then diverged. When MCM was the sole source of protein, marked curvature was seen as the response approached R_{\max} . The curves describing the response of rats fed diets containing the 60 CGM-40 MCM and 50 CGM-50 MCM mixtures were identical.

When nitrogen gain was the dependent variable a common value for parameters b and R_{\max} could again be shared by all curves (Table 3). Unlike that seen with weight gain, a unique value for parameter c was required to describe each curve adequately when nitrogen gain was the dependent variable for rats fed the diets containing the three CGM-MCM protein mixtures. Even though a common value for parameter d could be used in the model to describe the response of rats fed diets containing the three different CGM-MCM protein mixtures, none of the curves were identical because of the differences in parameter c .

The five dose-response curves fitted to the nitrogen gain-nitrogen intake data are shown in Fig. 2. There were differences in the shape (parameter c) of the curves describing the responses of rats fed diets containing the three CGM-MCM mixtures. No differences were evident in scaling (parameter d) with respect to the x-axis. Increasing the MCM in CGM-MCM protein mixtures resulted in steeper slopes.

Final parameter estimates for the fit of the model describing the effect of methionine supplementation of MCM when weight gain or nitrogen gain was the dependent variable are seen in Tables 4 and 5, respec-

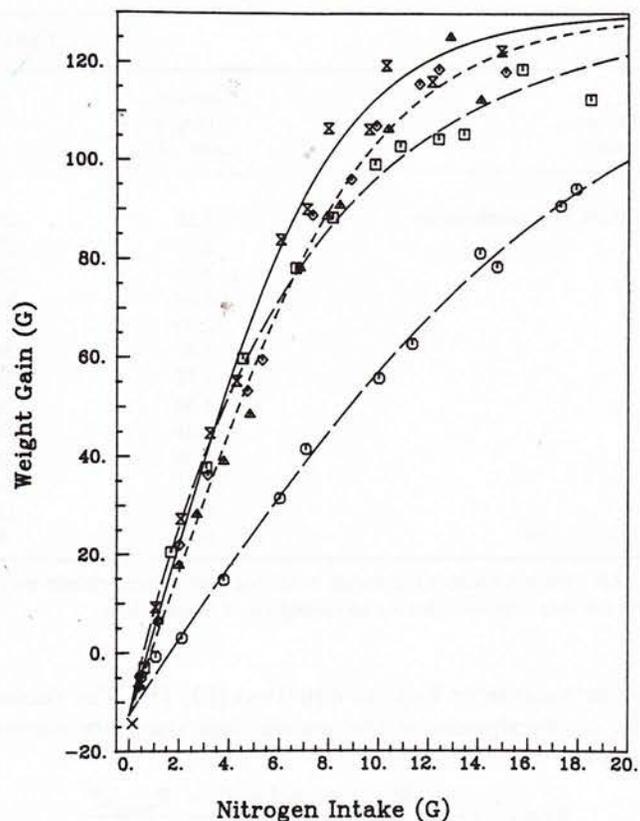


FIGURE 1 Body weight gain (g) vs. nitrogen intake (g) over 21 d for groups of four rats fed graded levels of corn gluten meal (CGM), Mormon cricket meal (MCM) or CGM-MCM mixtures. CGM (\circ — \circ), MCM (\square — \square), 60 CGM-40 MCM (\triangle — \triangle), 50 CGM-50 MCM (\diamond — \diamond), 40 CGM-60 MCM (\times — \times). Lines are the best fits to the data using the logistic model.

tively. In both cases the models could share a common value only for parameters b and R_{\max} . The fit of these curves to the data can be seen in Figs. 3 and 4. The response of rats fed diets containing MCM with and without supplemental methionine were similar at low levels of nitrogen intake but diverged as nitrogen intake increased.

One method to evaluate the quality of a protein is to determine the nitrogen intake required to achieve a

TABLE 3

Final parameter estimates of the nitrogen gain-nitrogen intake response curves for rats fed diets containing corn gluten (CGM), Mormon cricket meal (MCM) or CGM-MCM mixtures as a source of dietary protein

Protein source	Parameter ¹			
	b	c	d	R_{\max}
CGM	-0.263 ± 0.027	0.815 ± 0.399	0.925 ± 0.011	4.334 ± 0.091
60 CGM-40 MCM	-0.263 ± 0.027	3.397 ± 0.550	0.779 ± 0.013	4.334 ± 0.091
50 CGM-50 MCM	-0.263 ± 0.027	2.952 ± 0.481	0.779 ± 0.013	4.334 ± 0.091
40 CGM-60 MCM	-0.263 ± 0.027	1.910 ± 0.324	0.779 ± 0.013	4.334 ± 0.091
MCM	-0.263 ± 0.027	0.862 ± 0.314	0.833 ± 0.017	4.334 ± 0.091

¹ b = estimated nitrogen gain of rats fed the protein-free diet for 21 d; R_{\max} = estimated maximum 21-d nitrogen gain; mean \pm SD.

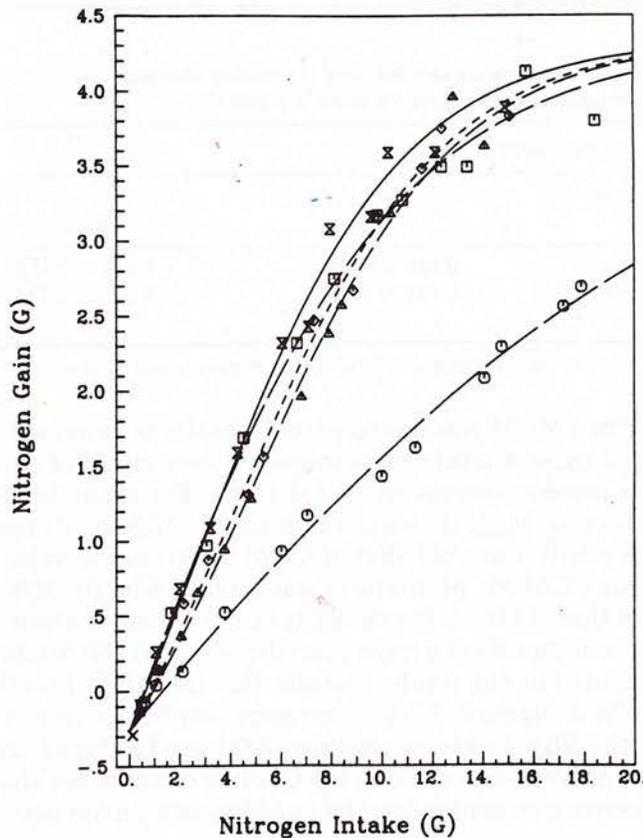


FIGURE 2 Body nitrogen gain (g) vs. nitrogen intake (g) over 21 d for groups of four rats fed graded levels of corn gluten meal (CGM), Mormon cricket meal (MCM) or CGM-MCM mixtures. CGM (○—○), MCM (□—□), 60 CGM-40 MCM (△—△), 50 CGM-50 MCM (◇—◇), 40 CGM-60 MCM (X—X). Lines are the best fits to the data using the logistic model.

specific response (12, 15, 17). The nitrogen intakes and relative values of each of the five protein sources are compared at 0, 50 and 95% of the R_{max} in Table 6. The relative value was calculated as the nitrogen intake required to achieve a specific weight, or nitrogen gain divided by the nitrogen intake required to achieve an identical response by rats fed diets containing the 40 CGM-60 MCM diet that was used as the control. All calculations are based on the best-fit model with the parameter estimates from Tables 2 and 3.

When body weight gain was the dependent variable two distinct patterns emerged. A constant relative value of 84% was seen from maintenance to 95% of the R_{max} when rats were fed diets containing the two other CGM-MCM protein mixtures. In contrast, a consistently declining relative value was seen as the response increased from maintenance to 95% of the R_{max} when rats were fed diets containing either MCM or CGM as the sole protein source. What is especially noteworthy is that the relative value of the diets containing MCM was superior to that of the control when used for maintenance but was inferior to the control when used to support near maximum growth (95% of the R_{max}).

A slightly different pattern was seen when nitrogen gain was the dependent variable. Again a consistently declining relative value was seen as the response increased from maintenance to 95% of the R_{max} when rats were fed diets containing either CGM or MCM as the sole source of protein. The pattern of the response of rats fed diets containing the two other CGM-MCM protein mixtures was such that a consistently increasing relative value was seen from 0 to 95% of the R_{max} .

The nitrogen intakes and relative values of MCM with and without supplemental methionine are shown in Table 7. The relative response of rats fed diets containing MCM without methionine consistently decreased relative to that of rats fed diets containing MCM supplemented with methionine. Although the patterns observed were similar regardless of the response criteria, when nitrogen gain was the dependent variable, the magnitude of the decrease was less than that seen when weight gain was the dependent variable.

DISCUSSION

The results reported here show that ground Mormon crickets are a suitable source of protein when fed to rats in purified diets. The quality of MCM protein is moderate and promotes maximum weight and nitrogen gains at dietary protein levels of approximately 30%. The results also indicate that methionine was the first limiting amino acid for growth and that when supplied either by a complementary protein source (CGM) or by

TABLE 4

Final parameter estimates of the weight gain-nitrogen intake response curves for rats fed diets containing Mormon cricket meal (MCM) with and without supplemental methionine as the sole source of dietary protein

Protein source	Parameter ¹			
	<i>b</i>	<i>c</i>	<i>d</i>	R_{max}
MCM	-14.032 ± 1.785	0.114 ± 0.270	0.837 ± 0.026	123.187 ± 3.251
MCM plus 0.4% methionine	-14.032 ± 1.785	2.284 ± 0.679	0.642 ± 0.035	123.187 ± 3.251

¹*b* = estimated weight gain of rats fed the protein-free diet for 21 d; R_{max} = estimated maximum 21-d weight gain; mean ± SD.

TABLE 5

Final parameter estimates of the nitrogen gain–nitrogen intake response curves for rats fed diets containing Mormon cricket meal (MCM) with and without supplemental methionine as the sole source of dietary protein

Protein source	Parameter ¹			
	<i>b</i>	<i>c</i>	<i>d</i>	<i>R</i> _{max}
MCM	-0.342 ± 0.053	0.835 ± 0.416	0.826 ± 0.025	4.243 ± 0.133
MCM plus 0.4% methionine	-0.342 ± 0.053	2.238 ± 0.679	0.701 ± 0.031	4.243 ± 0.133

¹*b* = estimated nitrogen in gain of rats fed the protein-free diet for 21 d; *R*_{max} = estimated maximum 21-d nitrogen gain; mean + SD.

purified methionine, the nutritional value of the mixture is increased. Interestingly, the protein quality of MCM when used for maintenance was equal or superior to that of any other protein source tested. These results are important because they indicate that methionine is probably not the first limiting amino acid when MCM is used for maintenance, but it is clearly the first limiting amino acid when MCM is used for growth. This supports the suggestion that the difference in the protein required for growth and maintenance is not just a decrease in the amount of dietary protein but also a change in the amino acid patterns, (18).

When MCM was mixed with CGM the protein quality of these mixtures was improved over either of the two protein sources when fed alone. For example at 95% of the *R*_{max}, the value of the CGM-MCM mixtures was nearly threefold that of CGM, whereas the value of the CGM-MCM mixtures was increased by 10–20% over that of MCM. On the basis of amino acid analysis, the best diet should have been the 50 CGM–50 MCM mixture but our results indicate that the 40 CGM–60 MCM is superior. This discrepancy may be due in part to the high levels of choline, 4900 mg/kg, found in ground Mormon crickets (10). Choline can enhance the effective methionine level by providing one-carbon units

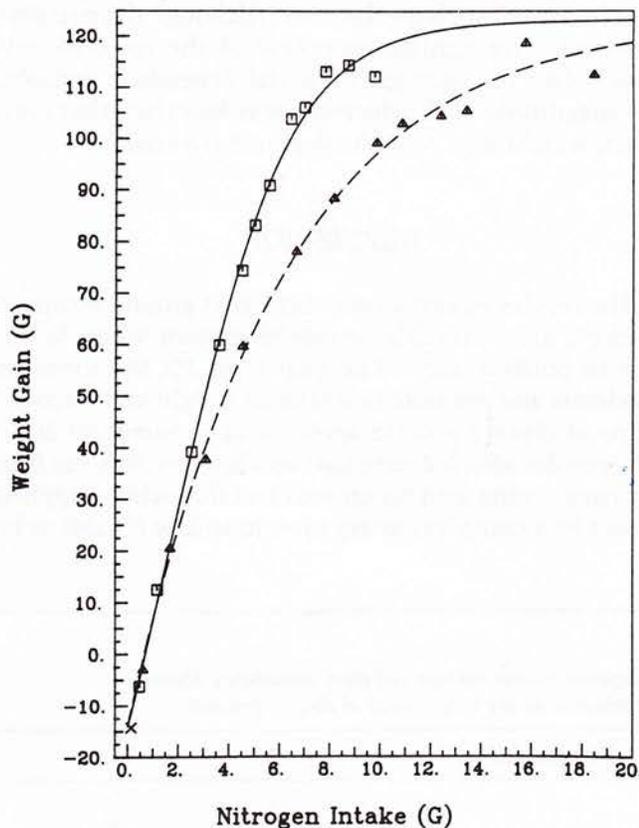


FIGURE 3 Body weight gain (g) vs. nitrogen intake (g) over 21 d for groups of four rats fed graded levels of Mormon cricket meal (Δ — — Δ) or Mormon cricket meal supplemented with 0.4% of L-methionine (\square — — \square). Lines are the best fits to the data using the logistic model.

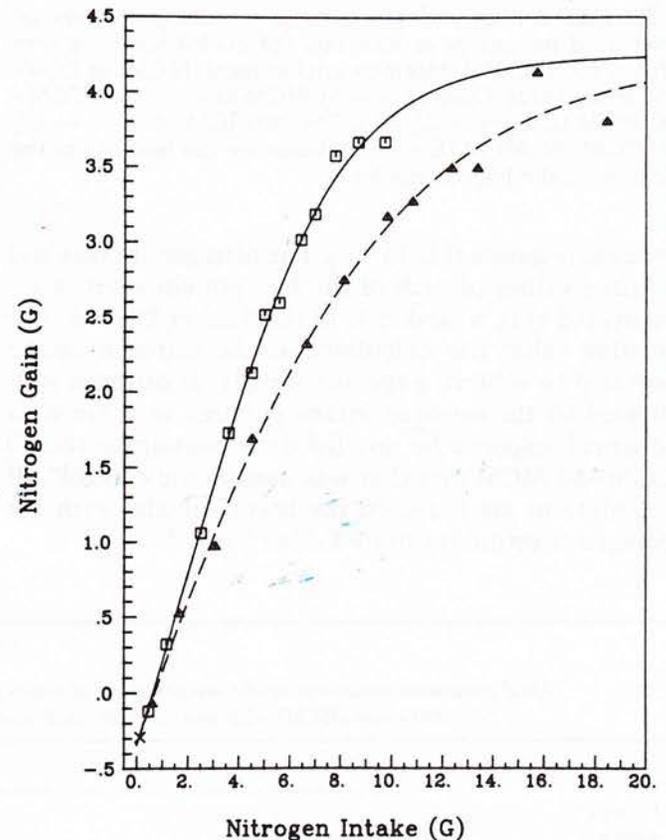


FIGURE 4 Body nitrogen gain (g) vs. nitrogen intake (g) over 21 d for groups of four rats fed graded levels of Mormon cricket meal (Δ — — Δ) or Mormon cricket meal supplemented with 0.4% of L-methionine (\square — — \square). Lines are the best fits to the data using the logistic model.

TABLE 6

Projected nitrogen intake (g/21 d) and relative values required for 0, 50 and 95% of the maximum weight and nitrogen gains

Protein source	Percent of the maximum body weight gain			Percent of the maximum nitrogen gain		
	0	50	95	0	50	95
CGM						
Nitrogen intake, g	1.7	11.4	35.1	1.4	14.2	46.4
Relative value, %	45	41	38	48	41	35
60 CGM-40 MCM						
Nitrogen intake, g	0.9	5.6	15.7	1.0	7.2	18.1
Relative value, %	84	84	84	69	81	91
50 CGM-50 MCM						
Nitrogen intake, g	0.9	5.6	15.7	0.9	6.8	17.7
Relative value, %	84	84	84	76	86	93
40 CGM-60 MCM						
Nitrogen intake, g	0.8	4.7	13.3	0.7	5.8	16.5
Relative value, %	100	100	100	100	100	100
MCM						
Nitrogen intake, g	0.6	5.2	21.9	0.69	6.2	20.0
Relative value, %	128	90	61	111	94	82

for transmethylation reactions that convert homocysteine to methionine (19).

When MCM was supplemented with methionine the protein quality of the MCM-methionine mixture was increased above that seen for any of the CGM-MCM protein mixtures. Probably the most important point is the 40-50% decrease in the amount of protein required for rats to reach 95% of the R_{max} when supplemental methionine was added to MCM. Rats fed diets containing MCM supplemented with methionine attained maximum weight gains at dietary protein concentrations around 16%. The protein requirement (as ideal protein) for growth as recommended by the National Research Council is 12%, which shows the high quality of MCM protein when supplemented with methionine (19).

Variation in the shape of the dose-response curves adds another dimension to the analysis of the dose-response relationship. The changes in the relative values seen in Table 6 clearly show that differences exist in the shape of the dose-response curves, but the most

dramatic example can be seen in Fig. 4. The curve describing the response of rats fed diets containing MCM without methionine shows a great deal of curvature, whereas the curve describing the response of rats fed diets containing MCM supplemented with methionine shows much less curvature. This difference can be demonstrated mathematically by determining the slopes of the two curves at various points along the curve (Table 8). Note that the slopes for the curve describing the response of rats fed diets containing MCM supplemented with methionine are almost constant up to approximately 50% of the R_{max} , indicating an almost linear response, and then decline slowly thereafter. In contrast, the slopes for the curve describing the response of rats fed diets containing MCM show a continual decrease. This decrease in efficiency of nutrient utilization as the response approaches R_{max} has been referred to by Parks as the "area of diminishing returns" (20).

Because the magnitude of the diminishing returns component appears to be highly variable there may be

TABLE 7

Projected nitrogen intake (g/21 d) and relative values required for 0, 50 and 95% of the maximum weight and nitrogen gains

Protein source	Percent of the maximum body weight gain			Percent of the maximum nitrogen gain		
	0	50	95	0	50	95
MCM plus 0.4% methionine						
Nitrogen intake, g	0.7	3.6	9.6	0.7	4.4	11.8
Relative value, %	100	100	100	100	100	100
MCM						
Nitrogen intake, g	0.7	4.8	18.0	0.7	6.0	19.1
Relative value, %	106	75	53	90	74	62

TABLE 8
Slopes at the specified responses using the logistic equation
and the parameter estimates shown in Table 4

Response (percent of R_{max})	Slope (N gain/N intake)	
	MCM	MCM with methionine
0	19.91	20.50
10	18.10	21.52
20	16.25	21.86
30	14.36	21.51
40	12.43	20.48
50	10.46	18.77
60	8.44	16.38
70	6.39	13.31
80	4.30	9.55
90	2.17	5.10

practical applications for dose-response curves. Most applied feeding programs are designed to maximize growth rate at the cost of reduced feeding efficiency. For responses that show a great deal of curvature, the incremental increase in growth obtained by an increase in the dietary nutrient concentration may not be economically justified.

One factor that has an effect on the shape of the dose-response curve is the choice of the independent variable. Velu, Baker and Scott (21) have suggested that when the response is analyzed as a function of dietary nutrient concentration the pattern is curvilinear, whereas using nutrient intake as the independent variable results in a linear relationship. An inspection of the data from the experiments reported here as well as those from a number of other papers clearly shows that a curvilinear response is not purely the result of the independent variable (21–24). Voluntary food intake has clearly been shown to be a function of dietary protein concentration (25); thus, the choice of the independent variable would have an effect on the shape of the curve, although it is definitely not the sole reason for a curvilinear response. In the studies reported here, nutrient intake was used as the independent variable because the actual dose an animal receives is a function of both the dietary nutrient concentration and food intake.

The choice of the independent variable selected should clearly reflect the experimental hypothesis tested. When dietary nutrient concentration is used as the independent variable, the results obtained reflect both the metabolic effects of the nutrient as well as the effect of the nutrient on voluntary food intake. In contrast, when nutrient intake is used as the independent variable, variations in food intake are accounted for and the resulting relationship reflects metabolic responses to the nutrient being tested. Two separate analyses of nutrient-response interactions using both dietary nutrient concentration and nutrient intake as the independent variable may provide a unique way of separating differences caused solely by metabolic effects from those that involve differences in voluntary food intake.

Another factor affecting the shape of the dose-response curve is the choice of the dependent variable. As noted earlier, the pattern of the response varied depending on the response criteria selected, weight gain or nitrogen gain. The fact that this has an effect on the calculated value of the protein source suggests that body composition is affected by dietary protein quality as well as by dietary protein concentration (26). The effect of dietary protein concentration on carcass protein content is well documented (21, 27, 28). Carcass protein content (in percent) appears to be highest at both high and low dietary protein concentrations, reaching a nadir at intermediate protein concentrations. Because this pattern is not linear, the shape of the dose-response curve also depends upon the dependent variable selected. Although nitrogen gain is more difficult to measure than body weight gain, it is clearly a more reliable measure of dietary protein quality.

The third factor that has been shown to have an effect on the shape of the dose-response curve is the amino acid that is first limiting. The difference in the response of animals fed diets equally deficient in each of the essential amino acids has been shown to be markedly different by a number of researchers using both rats and poultry (29–31). Although Chu and Hegsted (32) attributed these response differences to differences in the activities of the amino acid-degrading enzymes, results from Okumura and Muramatsu (33) suggest that food intake is also involved. A consequence of these differences has been observed when rats were fed diets containing graded levels of proteins that were first limiting in lysine. At low levels of intake the curvature seen in the dose-response relationship of diets that contain proteins that are first limiting in lysine has proven to be a problem when evaluating protein quality with the slope-ratio technique (34, 35). Results obtained with amino acid mixtures limiting in either lysine or threonine, fed at 60–80% of the requirement, indicate that the identity of the limiting amino acid has a marked effect on both the weight gain and feed efficiency of rats (36). Sugahara, Baker and Scott have shown a similar effect for a number of the essential amino acids when amino acid diets were fed to chicks at 60% of the requirement (29). These results suggest that the identity of the limiting amino acid may affect the shape of the dose-response curve at higher levels of intake as well. It should be noted, however, that an accurate assessment of the amino acid requirement is crucial to formulate diets equally limiting in the essential amino acids.

A fourth factor that may have an effect on the shape of the dose-response curve is amino acid balance. Although a variety of studies have shown that excesses of the other amino acids have a pronounced effect on growth and nitrogen retention, most of this effect appears to be related to differences in voluntary food intake (37–39). This would have a substantial impact on the shape of the dose-response curve when the nutrient

dietary concentration is used as the independent variable, but using nutrient intake as the independent variable would tend to correct for alterations in food intake.

The variety of shapes in dose-response curves seen in these experiments raises a serious question on the use of single-point protein quality assays. These assays evaluate protein quality at a predetermined dietary protein concentration; hence their validity in determining the value of a protein source for maintenance or growth is suspect (12). In contrast, dose-response curves allow for the determination of the relative value of protein sources at any performance level. Because it has been shown that dose-response curves have different shapes, there is no one method for extrapolating the results of a single-point assay to growth levels other than those obtained in the single-point assay.

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