Use of a Four-Parameter Logistic Model to Evaluate the Quality of the Protein from Three Insect Species when Fed to Rats[1]

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

Departments of †Nutritional Science, *Entomology, and ‡Meat and Animal Science, University of Wisconsin, Madison, WI 53706

ABSTRACT The quality of three insect protein sources [Mormon cricket meal (MCM), house cricket meal (HCM) and Eastern tent caterpillar meal (TCM)] was evaluated relative to that of lactalbumin (LA) and soy protein (SP) by using both amino acid analysis and a rat bioassay. The amino acid pattern of the three insect meals indicated that methionine should be the first limiting amino acid for growing rats. In the rat bioassay, weanling Sprague-Dawley rats were fed graded levels of the five proteins in purified diets and the response (weight or nitrogen gain) evaluated as a function of nitrogen intake. The individual nitrogen intake–animal response results could be described by a series of curves using a four-parameter logistic model. The use of parameter sharing permitted the full range of responses to be described so that statistical differences between the dose-response curves could be identified. When used for either weight maintenance, nitrogen equilibrium, maximum weight gain or maximum nitrogen retention, the five protein sources could be ranked in the following order: LA > HCM > MCM = SP > TCM. Relative to lactalbumin, the value of all four protein sources decreased with increasing nitrogen intake. The low values obtained for TCM may have been related to factors other than protein quality. The results of this study indicate that some insect proteins are equivalent or superior to soy protein as a source of amino acids for growing rats. *J. Nutr.* 119: 864–871, 1989.

INDEXING KEY WORDS:

• *lactalbumin* • *soy protein* • *insect protein* • *protein quality* • *nonlinear models* • *logistic model* • *parameter sharing*

Since the 1960s, results from a number of studies designed to investigate the nutritional value of insect protein have been published. House fly (*Musca domestica* L.) pupae (1–3), face fly (*Musca autumnalis* DeGeer) pupae (4), Mormon crickets (*Anabrus simplex* Haldeman) (5, 6), house crickets (*Acheta domesticus* L.) (7) and a variety of lepidoptera species (8–10) have been evaluated as a source of protein in poultry diets. In general, these studies indicated that insect protein was a suitable substitute for soybean meal in practical poultry diets. Amino acid analyses of insect protein indicated that the sulfur amino acids, methionine and cysteine, are probably the first limiting amino acids for poultry. However, when Mormon crickets or house crickets were used as a source of protein in purified diets for poultry, methionine and arginine were found to be co-limiting (6, 7).

We know of only three studies designed to evaluate the value of an insect species when fed to animals other than poultry. In the first study, the quality of protein from the termite species, *Macrotermes falciger*, was evaluated in a rat bioassay using the PER method, and a value of 1.7 was obtained (11). The low value for termite protein was attributed to its low digestibility (51%) relative to that of casein (84%). The high temperatures used in the processing of the insect material may have altered its protein quality by decreasing amino acid availability. In another study, soldier fly larvae, *Hermetia illucens*, were incorporated into a practical-type diet and fed to pigs. Dry matter digestibility was significantly lower for this diet than for a corn-soybean meal control diet (12). In the third study, dried, ground ether-extracted Mormon crickets were used as a protein source in a multi-dose rat bioassay (13). When the Mormon cricket meal diets were supplemented with 0.4% L-methionine, the amount of crude protein required to attain 95% of the maximal growth rate or nitrogen retention was reduced by 45%. When the Mormon cricket meal was mixed with a complementary protein source, corn gluten meal, the amount of crude protein required to attain 95% of the maximal growth or nitrogen retention was reduced by approximately 30% relative to Mormon cricket meal alone.

Because of the encouraging results obtained with

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Mormon cricket meal (13), the present investigation was conducted to compare the quality of protein of three insect species, the Mormon cricket, the house cricket and the Eastern tent caterpillar (*Mallacosoma americanus* Fab.), with that of lactalbumin and isolated soy protein. The five protein sources were compared using both amino acid analyses and a rat bioassay procedure described earlier (14). A nonlinear model was used to describe the dose-response curves, and the relative values of the proteins were evaluated by comparing the amount of nitrogen required to achieve identical levels of performance (13, 14).

MATERIALS AND METHODS

Protein sources. The lactalbumin and isolated soy protein were purchased commercially (Teklad Test Diets, Madison, WI). Adult Mormon crickets were collected and processed as described earlier (13). Adult house crickets were obtained from a colony maintained at the University of Wisconsin, Department of Entomology, and killed by freezing. Final and penultimate instar Eastern tent caterpillar larvae were obtained from multiple locations in Dane County, WI and killed by freezing. All insects used in these experiments were kept frozen until needed.

The lactalbumin and isolated soy protein were used as purchased. The Mormon crickets and house crickets were dried by lyophilization. The Eastern tent caterpillar larvae were dried in a radiant heat oven at 45°C for 72 h to singe off the body hairs. After drying, each insect preparation was ground through a 1-mm (approximately) screen in a Wiley mill. The lipid material was removed by refluxing with diethyl ether for 12 h, and the dry powder was redried at 45°C for 6 h to remove any residual ether. All protein sources were passed through a 40-mesh screen prior to use.

Each of the protein sources was analyzed for ash, water and acid detergent fiber using procedures established by the A.O.A.C. (15). Ether extract was determined by a 24-h Soxhlet extraction using diethyl ether. Nitrogen was determined by a micro-Kjeldahl digestion procedure with ammonia determined by a phenol-hypochlorite reaction (16).

The amino acid concentration was determined in a 40-μg sample. The sample was placed in an acid-washed ignition tube containing 200 μl of 6 *N* HCl with 0.2% phenol, the tube evacuated to 25 mTorr, hydrolized for 20 h at 110°C, evaporated to dryness and then redissolved in a 0.15 *N* lithium citrate buffer solution at pH 2.2 (17). The sample was then centrifuged [12,000 × *g* for 10 min at room temperature (21°C)] and a sample of the supernatant used for analysis (Beckman model 119 amino acid analyzer, Palo Alto, CA).

Diets. All diets contained a vitamin mix, 0.5% (18); a mineral mix (Teklad Test Diets, Madison, WI), 5.0% (18); corn oil (Archer Daniels Midland, Decatur, IL), 5.0%; choline chloride (U.S. Biochemical, Cleveland, OH), 0.2%; cellulose (U.S. Biochemical), 0.2% (used as a carrier for the choline choloride); and equal weights of cornstarch (Staley, Decatur, IL) and glucose monohydrate (Cerelose, Corn Products (CPC) International, Englewood Cliffs, NJ). The protein sources were added to the diet at the expense of the cornstarch-glucose mixture. While the mineral mix used has been shown to contain an inadequate level of zinc when used with amino acid diets, the possibility of zinc limiting growth is unlikely in this experiment, since both lactalbumin and soy protein contain adequate levels of zinc (52 and 39 mg/kg, respectively). In general, the dried, ground insect species used in these experiments have been shown to contain high levels of zinc (7, 19, unpublished data); Mormon cricket, 130 mg/kg; house cricket, 250 mg/kg; and Eastern tent caterpillar, 80 mg/kg.

The five protein sources were evaluated using a rat bioassay in a 3-wk experiment (13, 14). Six dietary levels of lactalbumin were used, while seven levels were used for the other four protein sources. A diet containing no added protein was formulated to serve as a baseline control. The dietary protein concentrations selected were not the same for all protein sources tested and were based on the amino acid composition of the test proteins. These levels were selected in an attempt to provide diets which would support a full range of animal responses, from maximum weight loss to maximum weight gain. The diets were fed in powdered form in metal feed cups. Diet and water were available ad libitum.

Animals. Weanling male Sprague-Dawley rats (21-d-old, Sprague-Dawley Farms, Madison, WI) were divided into 36 groups such that the mean weights of the groups were similar (mean 51.5 g; range 47–58 g). Groups of five rats were assigned to each of the 35 dietary treatments. In addition, one group was killed at the beginning of the experiment to obtain an estimate of body nitrogen at the time the rats were assigned to the treatment groups. Rats were kept in a room maintained at 25°C, with controlled lighting (12-h light/dark cycle) and housed individually in stainless steel wire-bottomed cages. Rats were weighed daily, and food intake was determined three times per week over the 21-d experiment. At the end of the experiment, the rats were killed by ether inhalation, 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 kept 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. 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 (16). All nitrogen determinations were done in triplicate. Nitrogen gain was calculated for each rat by subtracting the average calculated nitrogen content of five rats at the start of the experiment from that obtained from analysis of each rat at the end of the experiment.

Logistic model. The data from this experiment were analyzed using a four-parameter logistic equation (13, 14, 20). The model[2] 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 = predicted response of rats fed a protein-free diet (g/21 d)

c = shaping parameter

d = scaling parameter

R_{max} = predicted maximum response (g/21 d)

I = nitrogen intake (g/21 d)

All dose-response curves (weight or nitrogen gain vs. nitrogen intake) were fitted using a simultaneous curve-fitting routine previously described (13, 14, 21). 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.

RESULTS

The composition of the five protein sources tested in these experiments is shown in **Table 1**. The high content of acid detergent fiber (ADF) in the insect meals is of interest because some of the nitrogen is probably in the form of chitin (*N*-acetylglucosamine) (22). The nitrogen content of chitin (6.1%) is similar to the nitrogen content of the ADF residue of the three insect meals (5.0, 7.6 and 5.2% for MCM, HCM and TCM, respectively). If this nitrogen represents nonprotein nitrogen, then 5.3, 5.5 and 7.0% of the total nitrogen in MCM, HCM and TCM, respectively, is nonprotein nitrogen. Thus, animal response per unit of nitrogen consumed should be less for rats fed diets containing MCM, HCM or TCM based solely on dilution.

The indispensable amino acid content of lactalbumin was higher than that of all of the other protein sources. The low sulfur amino acid content of the other protein sources indicates that their value should be substantially less than that of lactalbumin.

The dietary nitrogen content, food intake, weight gain and nitrogen gain of each treatment group are shown in **Table 2**. Rats fed HCM clearly reached a plateau for weight gain, although none of the animals clearly reached a plateau for nitrogen retention. Diets containing TCM appeared to be less palatable than the other diets, especially at high dietary nitrogen concentrations.

The final parameter estimates for the fit of the model to the data when weight gain was the dependent variable are shown in **Table 3**. All curves were able to share a common parameter estimate for both b and R_{max}. Unique estimates for parameters c and d were obtained for the curves describing the response of rats fed lactalbumin and TCM. This indicates that both the shape and magnitude of the response of rats fed diets containing these two protein sources differed from that of rats fed diets containing the other three protein sources. The curves describing the response of rats fed diets containing soy protein, MCM or HCM shared a common value for parameter c, while the curves describing the response of rats fed diets containing soy protein and MCM shared a common estimate for all four parameters. **Figure 1** shows the relationship of the five curves to the data when the body weight gain was the dependent variable. The response of rats fed diets containing HCM could be described by a curve with an identical shape (parameter c) to that of rats fed diets containing soy protein or MCM, although the slope of the curve (parameter d) was slightly steeper. The curve describing the response of rats fed diets containing TCM had the least slope, while that for rats fed lactalbumin had the steepest slope. The fit of the curves to the data when nitrogen gain was the dependent variable is shown in **Figure 2**. As was seen when weight gain was the response variable, the curve describing the nitrogen intake–nitrogen gain relationship of rats fed diets containing lactalbumin had the steepest slope, while the curve describing the response of rats fed diets containing TCM had the least slope. The shape (parameter c) of the curves describing the response of rats fed diets containing HCM was identical to that for the curves describing the response of rats fed diets containing soy protein and MCM, but the slope (lower value for parameter d) was slightly steeper.

Final parameter estimates for the fit of the model to the data when weight gain or nitrogen retention was the dependent variable are shown in **Tables 3** and **4**, respectively. Although the parameter estimates obtained were dependent upon the response criteria selected, the patterns observed were identical.

Table 5 shows the relative values of the four test

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[2]The original model (equation 1) can be modified to allow parameter I to retain the appropriate units (g nitrogen consumed/21 d). The conversion to the final model (equation 3) and a description of the parameters are shown below.

$$\text{response} = \frac{R_{max} + [b(1 + C] - R_{max}]d^I}{1 + cd^I} \qquad \text{(equation 1)}$$

since

$$d^I = \exp[(\ln d)I] \qquad \text{(equation 2)}$$

The final equation becomes

$$\text{response} = \frac{R_{max} + [b(1 + C) - R_{max}] \exp[(\ln d)I]}{1 + c \exp[(\ln d)I]} \qquad \text{(equation 3)}$$

We thank Steve Denham for this modification of the original equation.

TABLE 1

Composition of the protein sources used in the feeding experiments

	Protein source				
	LA	SP	MCM	HCM	TCM
			%		
Crude protein (6.25 × N)[1]	73.7	88.0	69.9	78.0	61.5
Ether extract[1]	7.1	2.1	3.0	2.6	2.2
Ash[1]	1.7	3.3	7.7	5.4	8.5
Water[1]	9.0	9.1	7.4	6.6	7.2
Acid detergent fiber[1]	0.5	0.4	11.7	9.1	13.2
			mg/g crude protein		
Alanine[2]	54	34	81	86	53
Arginine	41	68	63	78	40
Aspartic acid	100	85	54	74	72
Cystine	54	16	13	9	7
Glycine	22	36	54	59	40
Glutamic acid	152	155	104	112	89
Histidine	20	3	22	25	18
Isoleucine	46	34	37	40	28
Leucine	125	68	68	76	48
Lysine	85	52	54	59	44
Methionine	25	8	15	17	12
Phenylalanine	40	44	33	34	29
Proline	44	38	39	46	28
Serine	49	47	48	43	37
Threonine	61	35	43	43	32
Tyrosine	41	30	47	53	34
Tryptophan[3]	ND	ND	ND	ND	ND
Valine	54	34	49	57	36
Ammonia	16	14	20	18	14
Total indispensable amino acids	592	412	415	491	328
Total dispensable amino acids	421	395	380	420	319
Total amino acids	1013	807	795	911	647
First limiting amino acid[4]	His	Met-Cys	Met-Cys	Met-Cys	Met-Cys
Chemical score[4]	80	48	58	52	38

[1]Each value represents the mean of three analyses. Protein source of each diet is as follows: LA, lactalbumin; SP, soy protein; MCM, Mormon cricket meal; HCM, house cricket meal; TCM, Eastern tent caterpillar meal.

[2]All amino acid values are expressed as mg/g crude protein. For lactalbumin and soy protein, the values shown represent one analysis. For the three insect species, the values shown represent the mean of two analyses.

[3]Not determined.

[4]Using NRC requirements for growth (25), chemical score was calculated as: ([First limiting amino acid (mg/g protein)] ÷ [NRC requirement (mg/g protein)]) × 100.

protein sources when lactalbumin was used as the standard. The relative values are calculated by dividing the nitrogen intake required to achieve a specific weight or nitrogen gain for rats fed diets containing lactalbumin by the nitrogen intake required to achieve an identical response for rats fed diets containing the test protein. When weight gain was the dependent variable, the relative value of soy protein and MCM decreased from 73% at maintenance to 50% at 95% of the maximum predicted weight gain. The relative value of HCM and TCM also declined from highs of 89% and 56% at maintenance to lows of 61% and 25%, respectively, at 95% of the maximum predicted weight gain. When nitrogen gain was the dependent variable, the relative values of all four protein sources were less than those seen when weight gain was the dependent variable, although the patterns observed were identical.

DISCUSSION

Amino acid analysis indicated that the sulfur amino acids, methionine and cystine, are probably the first limiting amino acids in protein from MCM, HCM and TCM (Table 1). These results are similar to amino acid analyses of other insect species (1, 3, 6, 7, 10, 12). Feeding studies with rats have confirmed that methionine was the first limiting amino acid for growth in Mormon cricket meal (13) and that further additions of lysine and histidine had no effect (unpublished results). No

TABLE 2

Dietary nitrogen content and 21-d food intake, weight gain and nitrogen gain of rats fed diets containing the test protein sources[1]

Protein source	Dietary N	Food intake	Wt gain	N gain
	%		g	
LA	2.42	276.4 ± 9.8	124.8 ± 6.5	3.77 ± 0.21
	2.01	269.5 ± 0.9	108.0 ± 4.7	3.10 ± 0.14
	1.58	227.8 ± 20.4	71.0 ± 8.0	2.27 ± 0.36
	1.22	204.4 ± 16.8	45.6 ± 4.9	1.35 ± 0.12
	0.80	164.6 ± 7.4	19.8 ± 1.6	0.68 ± 0.05
	0.40	131.4 ± 9.3	2.2 ± 2.0	0.16 ± 0.04
SP	4.73	271.0 ± 6.6	124.0 ± 1.7	3.72 ± 0.10
	4.03	274.6 ± 4.2	123.4 ± 3.5	3.54 ± 0.12
	3.23	298.6 ± 6.9	107.0 ± 5.2	3.12 ± 0.13
	2.37	281.0 ± 10.6	82.6 ± 4.6	2.38 ± 0.16
	1.52	194.8 ± 1.9	38.0 ± 0.8	1.02 ± 0.03
	0.77	144.2 ± 5.4	6.6 ± 1.3	0.26 ± 0.03
	0.48	135.4 ± 7.8	−1.8 ± 1.3	0.02 ± 0.03
MCM	4.76	286.0 ± 5.3	120.8 ± 1.3	3.75 ± 0.12
	3.91	298.4 ± 5.4	117.8 ± 3.0	3.56 ± 0.12
	3.12	314.8 ± 8.4	110.8 ± 2.6	3.17 ± 0.10
	2.45	277.8 ± 5.9	79.6 ± 2.7	2.28 ± 0.05
	1.59	226.5 ± 7.7	41.0 ± 4.2	1.19 ± 0.09
	0.74	145.4 ± 4.9	6.0 ± 1.8	0.23 ± 0.05
	0.36	103.4 ± 5.8	−3.0 ± 1.6	−0.01 ± 0.03
HCM	4.69	290.6 ± 4.5	135.6 ± 2.8	4.20 ± 0.10
	3.97	297.2 ± 7.7	137.2 ± 4.6	3.96 ± 0.18
	3.18	300.4 ± 7.6	124.2 ± 4.1	3.55 ± 0.10
	2.50	309.6 ± 13.0	107.4 ± 4.8	2.88 ± 0.14
	1.64	254.4 ± 14.1	55.8 ± 4.4	1.47 ± 0.11
	0.82	145.6 ± 2.7	11.2 ± 0.7	0.30 ± 0.01
	0.45	115.6 ± 4.0	−2.6 ± 0.4	0.03 ± 0.01
TCM	4.79	211.0 ± 7.9	67.0 ± 6.0	2.04 ± 0.16
	4.00	229.8 ± 19.6	75.8 ± 9.4	2.01 ± 0.21
	3.18	188.2 ± 15.2	45.6 ± 7.4	1.36 ± 0.17
	2.47	194.2 ± 1.8	41.4 ± 1.3	1.14 ± 0.07
	1.58	169.2 ± 7.7	21.0 ± 2.8	0.58 ± 0.07
	0.78	121.4 ± 3.2	−3.2 ± 0.7	0.00 ± 0.02
	0.51	103.4 ± 3.7	−7.4 ± 1.2	−0.16 ± 0.02
Protein-free	0.00	93.6 ± 3.2	−12.2 ± 0.4	−0.28 ± 0.02

[1]Abbreviations for protein sources are described in footnote to Table 1. The values for rats fed the diet containing TCM at 4.79% N represent the means ± SEM, $n = 3$. The values for rats fed diets containing TCM at 4.00% N, and MCM at 1.59% N, represent the means ± SEM, $n = 4$. All other values represent the means ± SEM, $n = 5$.

feeding studies have yet been conducted to confirm that the sulfur amino acids are first limiting in HCM or TCM when fed to rats. When MCM or HCM was fed to young broiler chicks in purified diets, methionine and arginine appeared to be co-limiting (6, 7).

When compared to lactalbumin, the other four protein sources were inferior at all levels of animal performance (Table 5). Two of the three insect meals, MCM and HCM, were equal to and slightly superior to soy protein in the promotion of maximum growth or nitrogen gain. In contrast, the ability of TCM protein to promote maximum growth or nitrogen retention was only half that of soy protein. These results suggest that the nutritional quality of insect meal may depend upon the insect species. The reasons for the poor nutritional quality of TCM is unknown, but may be related to factors other than protein quality. Rats fed diets containing the two highest levels of TCM developed a pronounced diarrhea during the study. When these rats were killed at the end of the experiment and the gastrointestinal contents removed, it was noted that many of these rats had abnormally distended stomachs and colons. Two rats fed diets containing the highest level of TCM, and one rat fed the diet containing the second highest level of TCM, died after 1 wk.

In an attempt to compare the relative value of these proteins by utilizing the amino acid content of the proteins, a decision must be made on whether or not methionine or total sulfur amino acid content is used for evaluating proteins first limiting in the sulfur amino acids. Calculations based on total sulfur amino acids were used here because diets containing the same amount of sulfur amino acids (mmol sulphur/100 g diet) supported identical growth rates even though the amount of cystine varied from one half to over two thirds of the total (23, 24). Although not measured in this experiment, it was assumed that tryptophan was not the first limiting amino acid (7, 13).

TABLE 3

Final parameter estimates of the weight gain–nitrogen intake response curves of rats fed diets containing the test protein sources

Protein source	Parameter[1]			
	b	c	d	R_{max}
LA	−11.6 ± 0.9	2.567 ± 0.641	0.645 ± 0.032	152.6 ± 5.6
SP	−11.6 ± 0.9	1.006 ± 0.346	0.826 ± 0.021	152.6 ± 5.6
MCM	−11.6 ± 0.9	1.006 ± 0.346	0.826 ± 0.021	152.6 ± 5.6
HCM	−11.6 ± 0.9	1.006 ± 0.346	0.792 ± 0.026	152.6 ± 5.6
TCM	−11.6 ± 0.9	0.019 ± 0.400	0.926 ± 0.022	152.6 ± 5.6

[1]Abbreviations for protein sources are described in footnote to Table 1; b = estimated weight gain of rats fed the protein-free diet for 21 d; R_{max} = estimated maximum 21-d weight gain. All values represent means ± SE.

Using the amino acid requirements for growth (25), the chemical score was determined for each of the five protein sources (Table 1). A comparison of these values with the ranking of the four protein sources at 0, 50 and 95% of the maximum nitrogen gain (Table 5) revealed that the values based on amino acid analyses compared most favorably with those from the multi-dose assay when the values at 0% of R_{max} were used. The relative values based on amino acid analysis (60, soy protein; 73, MCM; 65, HCM; and 48, TCM) varied from 86 to 109% of that of the measured values at nitrogen equilibrium, and 116 to 229% at 95% of the maximum nitrogen gain. Thus, it appears that the quality of insect protein is as good as soy protein and, like soy protein, the values relative to lactalbumin decline with increasing concentration in the diet. With the exception of TCM, the relative value of all of these proteins declined at similar rates relative to lactalbumin. One wonders if this is a coincidence, or if the value of proteins limited by the same indispensable amino acid decline at the same rate.

One of the advantages of the multi-dose assay is the ability to compare the relative values of proteins across animal growth rates from 0 (maintenance) to 95% of the estimated maximal growth rate (Table 5). The value of the four test proteins, relative to that of lactalbumin, fell as nitrogen intake and nitrogen gain increased (Table 5). At maintenance (zero gain), the relative values of the test proteins (soy protein, MCM, HCM and TCM) varied from 50 to 76% that of lactalbumin. At 95% of

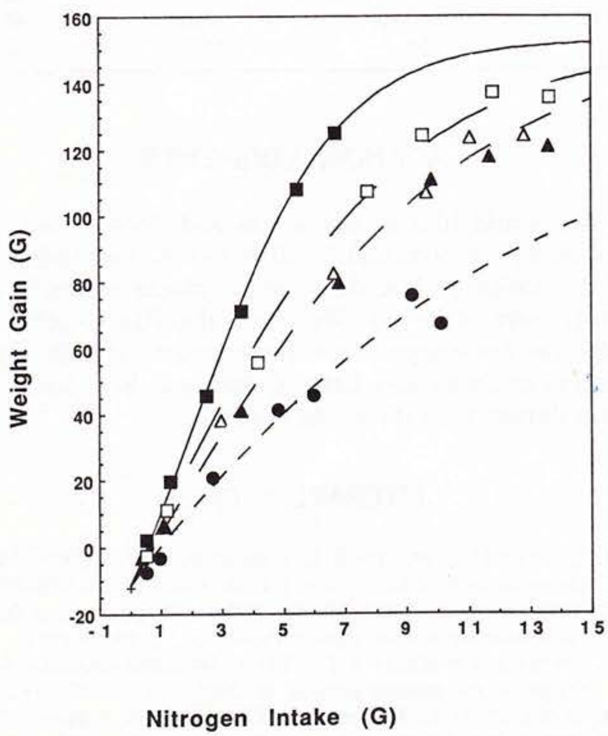

FIGURE 1 Body weight gain of rats fed graded levels of protein from one of five different protein sources. Lactalbumin (■), house cricket meal (□), soy protein (△), Mormon cricket meal (▲), Eastern tent caterpillar meal (●) and protein free (+). The lines are the best fits using the logistic model.

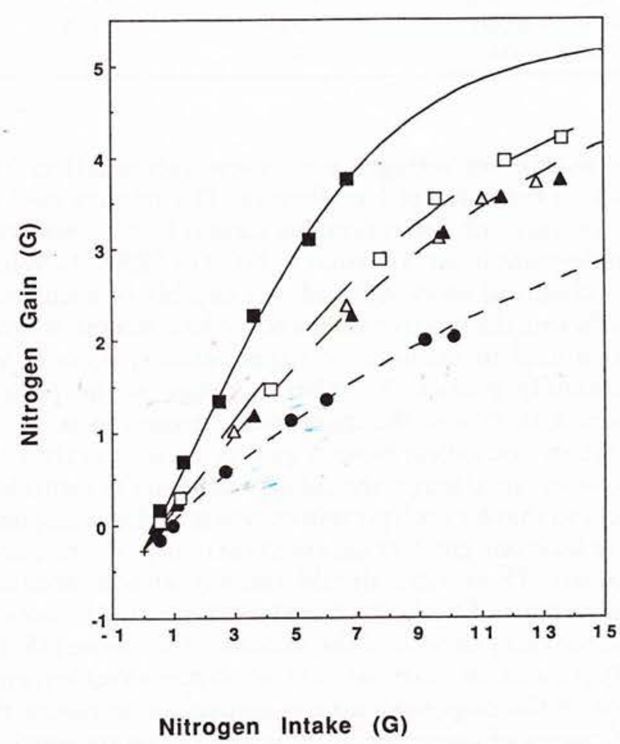

FIGURE 2 Nitrogen gain of rats fed graded levels of protein from one of five different protein sources. Lactalbumin (■), house cricket meal (□), soy protein (△), Mormon cricket meal (▲), Eastern tent caterpillar meal (●) and protein free (+). The lines are the best fits using the logistic model.

TABLE 4

Final parameter estimates of the nitrogen gain–nitrogen intake response curves of rats fed diets containing the test protein sources

	Parameter[1]			
Protein source	b	c	d	R_{max}
LA	−0.26 ± 0.03	1.733 ± 0.556	0.732 ± 0.042	5.29 ± 0.42
SP	−0.26 ± 0.03	0.765 ± 0.395	0.872 ± 0.027	5.29 ± 0.42
MCM	−0.26 ± 0.03	0.765 ± 0.395	0.872 ± 0.027	5.29 ± 0.42
HCM	−0.26 ± 0.03	0.765 ± 0.395	0.855 ± 0.031	5.29 ± 0.42
TCM	−0.26 ± 0.03	−0.270 ± 0.015	0.957 ± 0.025	5.29 ± 0.42

[1]Abbreviations for protein sources are described in footnote to Table 1; b = estimated nitrogen gain of rats fed the protein-free diet for 21 d; R_{max} = estimated maximum 21-d nitrogen gain. All values represent means ± SE.

TABLE 5

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

	Percent of the maximum body wt gain			Percent of the maximum N gain		
Protein source	0	50	95	0	50	95
LA: N intake	0.55	3.73	9.84	0.40	4.45	12.89
relative value	100	100	100	100	100	100
SP: N intake	0.74	6.27	19.61	0.61	7.88	26.24
relative value	73	59	50	67	56	49
MCM: N intake	0.74	6.27	19.61	0.61	7.88	26.24
relative value	73	59	50	67	56	49
HCM: N intake	0.61	5.14	16.07	0.53	6.89	22.95
relative value	89	72	61	76	65	56
TCM: N intake	0.97	10.10	40.17	0.80	13.38	62.41
relative value	56	37	25	50	33	21

the maximum nitrogen gain, these values fell to 21–56% of the value of lactalbumin. The relative decline in the value of insect proteins cannot be assessed with single-point bioassays such as NPU or PER (14). While the chemical score method was capable of accurately predicting the relative values of the four protein sources when used to maintain nitrogen balance, it could not accurately predict the relative values of the protein sources at 95% of the maximum nitrogen gain.

When evaluating protein quality using a multi-dose bioassay, an attempt should also be made to formulate diets so that for each protein source tested, the response of at least one group of animals is at or near the response plateau. These data should support a more accurate assessment of both the "diminishing returns" area of the response curve and the maximum response (13, 14, 26). An accurate estimate of the "diminishing returns" area of the response curve is critical in assessing the efficiency of incremental increases of dietary nutrient concentration as the response approaches R_{max}. This "area of diminishing returns" has been shown to vary widely between rats fed diets containing different dietary protein sources (14).

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