The Utilization of Nitrogen for Growth in Mice Fed Blends of Purified Proteins (42856)

MURRAY O. KEITH AND J. MILTON BELL
Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N OWO

Abstract. **Reasons for differences in growth of weanling mice fed different blends of purified proteins in diets apparently adequate in indispensable amino acids were investigated. Dietary nitrogen provided at increasing levels from a mixture of casein, gelatin, and zein, or from these plus gliadin and lactalbumin resulted in similar weight gains and efficiency of feed utilization in weanling mice. These responses were always lower ($P < 0.05$) than for mice fed the control diet. When diets were based on true digestible nitrogen and amino acids, weight gains and feed efficiency decreased ($P < 0.05$) as the dietary nitrogen level increased. High plasma glycine concentrations and high phenylalanine to tyrosine ratios were associated with high dietary glycine levels provided by gelatin. When a mixture of constant proportions of protein sources, predominantly casein, supplied most of the dietary nitrogen, small increases in weight gains and feed efficiency were observed as the nitrogen level in the diet increased. Varying the proportions of sources, which increased the percentage of gelatin, resulted in decreased gains in response to more dietary nitrogen. Replacing the amino acids found in gelatin by crystalline L-amino acids and replacing glycine by glutamic acid improved gains and feed efficiency ($P < 0.05$) compared with gelatin-containing diets. These studies demonstrated that glycine at dietary concentrations of approximately 1–2%, either from gelatin or as the free amino acid, inhibited growth and feed utilization of growing mice.** [P.S.E.B.M. 1989, Vol 190]

The requirements for indispensable amino acids (IAA) for mouse growth have been reported (1, 2) but the requirement for total dietary nitrogen has not been accurately defined. The dispensable amino acids (DAA), which supply nonspecific nitrogen if the IAA are provided at the requirement level, differ in nutritional value for simple-stomached animals (3, 4). For example, excessive dietary glycine can depress growth of rats (5, 6) and chicks (7).

Although casein is used extensively in experimental diets, several other commercially refined proteins may be used to extend the opportunity through linear programming to formulate diets for a wide range of amino acid (AA) specifications while minimizing the need for crystalline AA. The success of this approach depends upon having adequate knowledge of the composition, interchangeability, and digestibility of the alternative protein sources.

Received February 8, 1988. [P.S.E.B.M. 1989, Vol 190]
Accepted November 16, 1988.

0037-9727/89/1903-0246$2.00/0

The objective of this study was to examine the growth and feed efficiency (FE; gain/feed) responses of weanling mice fed diets containing IAA at requirement levels (1, 2) with various levels of total dietary nitrogen and with the DAA supplied by several blends of purified proteins and amino acids.

Materials and Methods

The effects of basing diet formulations on true digestible nitrogen and AA instead of total nitrogen and AA, of allowing unrestricted selection of protein sources compared with fixed or constant proportions when linear programming diets, of expanding the selection of protein sources, and of restricting the glycine level of the diet were successively investigated in a series of seven growth trials with weanling mice (Table I). All diets in Experiments 1–6 were formulated to minimize the use of crystalline IAA. In Experiments 3 and 4, DAA were supplied to meet the desired level of dietary nitrogen.

Diet formulation, excluding the nitrogen portion (Table II), was the same as in previous feeding trials with mice (1, 2). The American Institute of Nutrition (AIN) diet (8) was fed as a control diet. All diets were

Table I. Experimental Variables Used in Experiments 1–7

Experiment	Protein sources[a]	Proportions of protein sources	Assumed availability of nitrogen and AA	Dietary levels of nitrogen tested (g/kg)	AA added
1	Cas[a], gel, zein	Variable[b]	Total	14.8–21.6	IAA
2	Cas, gel, zein	Variable[b]	True digestible	14.4–21.8	IAA
3	Cas, gel, zein	Constant[c]	True digestible	14.5–21.1	IAA, DAA
4	Cas, gel, zein, glia, lact	Variable[b]	Total	15.2–21.8	IAA
5	Cas, gel, zein, glia, lact	Variable[b]	True digestible	14.9–22.3	IAA
6	Cas, gel, zein, glia, lact	Constant[c]	True digestible	14.3–20.4	IAA, DAA
7	Gel, zein		True digestible	20.6–21.6	IAA, DAA

[a] Cas, casein; gel, gelatin; glia, gliadin; and lact, lactalbumin.
[b] No restrictions in linear programming on use of protein sources among diets with different test levels of dietary nitrogen.
[c] Proportions of protein sources fixed according to the levels programmed in the lowest nitrogen diet of the preceding experiment.

Table II. Diet Formulas, Dry Matter Basis (g/kg)

Ingredient	Experimental diet[a]	Control diet[b]
Carbohydrate premix[c]	Variable	—
Protein and amino acids[d]	Variable	—
Sucrose	—	500
Cornstarch	—	140
Casein, high nitrogen[e]	—	200
Fat premix[f]	100	—
Corn oil	—	50
Cellulose-Celufil[e]	50	50
AIN mineral mix[b,e]	35	35
AIN vitamin mix[b]	10	10
Choline bitartrate[e]	2	2
DL-methionine	—	3
Antibiotic premix[g]	10	10

[a] As used by John and Bell (1) and Bell and John (2).
[b] Composition according to AIN (8).
[c] Composition: cornstarch, 50%; cerelose (glucose), 30%; sucrose, 20%.
[d] Replaced part of the carbohydrate premix; composition presented for individual experiments.
[e] Supplied by United States Biochemical Corp., Cleveland, OH.
[f] Composition: lard, 60.33%; sunflower oil, 39.42%; ethoxyquin (antioxidant), 0.25%.
[g] Tetralean 55 (MTC Pharmaceuticals, Hamilton, Ontario, Canada) in cornstarch (1:99), provided 5.5 mg tetracycline HCl/kg diet, omitted from Experiments 1 and 4.

formulated on a dry matter (DM) basis and adjusted to air-dry composition before mixing.

Two purified protein assortments, casein + gelatin + zein and the previous sources + gliadin + lactalbumin, supplied most of the nitrogen in the test diets. In an attempt to achieve maximum growth in weanling mice, a series of three experiments was conducted with each protein assortment using linear programmed diets designed to provide the minimum required concentrations of IAA but varying in nitrogen level. The recommended level of crude protein for growing mice is 12.5% (2.0% nitrogen × 6.25) (9) based on limited data.

Dietary concentrations of IAA were those recommended by John and Bell (1) and Bell and John (2) as follows (g/kg diet DM): histidine, 2.0; isoleucine, 3.7; leucine, 6.6; lysine, 4.0; methionine, 5.4; phenylalanine, 3.6; threonine, 4.5; tryptophan, 1.0; and valine, 5.4. In the estimation of the methionine requirement (1), the only cystine was a trace amount provided by the casein. The total phenylalanine plus tyrosine requirement was 6.6 g/kg (4 phenylalanine, 2.6 tyrosine), with all tyrosine being supplied by the protein sources (2). In the previous studies (1, 2) arginine was found to be dispensable and was considered a DAA in the present experiments. These concentrations of IAA were used for all experimental diets (control diet excluded) either on a total or true digestible basis as indicated for individual experiments. The mouse's requirements for AA such as asparagine, glutamic acid, and proline have not been determined. These AA are important for maximum growth in young rats (9) and, if of equal importance for growing mice, these AA could be assumed to have been provided by the 8–12% of various protein sources in the diets.

The first experiment in each series (Experiment 1, Table III and Experiment 4, Table IV) was based on chemically determined levels of total nitrogen and total IAA in the protein sources as reported previously (10). The diets were formulated to contain 14.4–20.8 g nitrogen/kg diet (9–13% crude protein, nitrogen × 6.25). In the second experiment in the series (Experiment 2, Table III and Experiment 5, Table IV), the diets provided true digestible nitrogen and IAA levels based on individual IAA digestibility coefficients also determined previously (10). True digestible nitrogen concentrations were 12.8–19.2 g/kg diet (8–12% true digestible crude protein). The proportional contribution of each protein source varied among diets in each of the four experiments.

In the third experiment in the series (Experiment 3, Table III and Experiment 6, Table IV) nitrogen and IAA were provided on a true digestible basis but the proportional contribution of each purified protein was held constant for all test diets within the experiment. This enabled the effects of increasing nitrogen concentration to be determined without possible confounding

Table III. Proportions of Nitrogen-Containing Components in the Diet Dry Matter of Experiments 1–3 Using Casein, Gelatin, and Zein as the Protein Sources (g/kg)

Diet	Total nitrogen by analysis[a]	True digestible nitrogen	Casein[b]	Gelatin[c]	Zein[d]	Crystalline amino acids	
						Indispensable	Dispensable[e]
Experiment 1							
1	14.8	—	39.9	27.0	17.8	8.0	—
2	16.5	—	33.6	40.4	19.0	8.2	—
3	18.1	—	27.3	53.7	20.2	8.3	—
4	19.8	—	21.0	67.1	21.3	8.5	—
5	21.6	—	14.6	80.4	22.5	8.6	—
Experiment 2							
6	14.4	12.8	68.6	3.8	10.1	7.4	—
7	15.8	14.4	53.5	23.5	14.0	8.6	—
8	18.4	16.0	37.2	43.8	18.3	10.1	—
9	20.4	17.6	17.6	65.4	24.2	12.1	—
10	21.8	19.2	0	84.8	29.5	13.9	1.0
Experiment 3							
11	14.5	12.8	68.6	3.8	10.1	7.4	0
12	16.0	14.4	68.6	3.8	10.1	7.4	14.0
13	17.8	16.0	68.6	3.8	10.1	7.4	28.0
14	19.2	17.6	68.6	3.8	10.1	7.4	42.0
15	21.1	19.2	68.6	3.8	10.1	7.4	56.0

[a] Total nitrogen by analysis in the control diet fed in Experiments 1–7 ranged from 30.0 to 31.5 g/kg dry matter.
[b] High nitrogen casein, United States Biochemical Corp, Cleveland, OH, supplier.
[c] Bloom 300, from porcine skin (United States Biochemical Corp.).
[d] ICN Nutritional Biochemicals, Cleveland, OH, supplier.
[e] Composition; % dry weight (Experiment 2) ala:gly:ser = 25:50:25, as used previously (1); (Experiment 3) ala:arg:glu:gly = 15:4:78:3.

Table IV. Proportions of Nitrogen-Containing Components in the Dry Matter of Experiments 4–6 Using Casein, Gelatin, Gliadin, Lactalbumin, and Zein as the Protein Sources (g/kg)

Diet	Total nitrogen by analysis	True digestible nitrogen	Casein	Gelatin	Gliadin[a]	Lactalbumin[b]	Zein	Crystalline amino acids	
								Indispensable	Dispensable[c]
Experiment 4									
16	15.2	—	24.2	11.5	24.5	20.0	6.6	6.6	—
17	16.9	—	9.1	23.2	28.2	29.0	6.1	6.9	—
18	18.5	—	—	37.3	27.8	31.4	7.6	7.2	—
19	19.9	—	—	54.8	20.8	24.1	11.9	7.7	—
20	21.8	—	—	72.2	14.0	16.8	16.2	8.1	—
Experiment 5									
21	14.9	12.8	71.1	—	3.6	—	7.9	6.7	—
22	16.4	14.4	45.9	13.7	15.3	9.3	6.8	7.4	—
23	18.0	16.0	15.7	29.6	26.2	20.9	6.7	8.6	—
24	20.1	17.6	—	55.3	19.1	18.7	14.3	10.7	—
25	22.3	19.2	—	84.8	—	—	29.5	13.9	1.0
Experiment 6									
26	14.3	12.8	71.1	—	3.6	—	7.9	6.7	—
27	15.5	14.4	71.1	—	3.6	—	7.9	6.7	13.5
28	17.5	16.0	71.1	—	3.6	—	7.9	6.7	27.0
29	18.8	17.6	71.1	—	3.6	—	7.9	6.7	40.4
30	20.4	19.2	71.1	—	3.6	—	7.9	6.7	53.9

[a] Crude, from wheat gluten (United States Biochemical Corp.)
[b] ICN.
[c] Composition: % dry weight (Experiment 5) ala:gly:ser = 25:50:25 (1); (Experiment 6) ala:arg:glu:gly = 15:4:78:3.

from compositional changes in the nitrogen component coincident with each increment in nitrogen in the previous experiments. The composition of the basal diet was the same as that diet having the lowest level of true digestible nitrogen in the preceding experiment of each series. Using a constant basal diet necessitated including a greater proportion of crystalline DAA to meet total nitrogen requirements. The composition of the DAA mixture was changed (Tables III and IV) to minimize dependence on glycine as a source of nitrogen. The

proportions of alanine, arginine, and glycine were similar to those in casein. The proportion of glutamic acid was similar to glutamic acid plus glutamine in casein.

A seventh and final experiment (Table V) was designed to examine the possibility that gelatin or glycine caused reduced mouse growth in the previous experiments. Diets were formulated on a true digestible nitrogen and IAA basis. The true digestible AA supplied by gelatin and zein were replaced in turn by crystalline AA (Diets 32 and 33, respectively) or, in the case of Diet 34, by crystalline AA with the glycine from gelatin replaced by glutamic acid.

Swiss × Carworth Farms no. 1 crossbred male weanling mice were used for all experiments except Experiment 7 in which CD1 Swiss mice were used. All were maintained in stock cages and fed a 20% casein-based pretest diet for 1 to 3 days before being randomly allotted to test diets at 18–21 days of age and weighing 10–11 g. There were 10 replicates per treatment group except 12 per treatment in Experiment 7.

The mice were kept in individual stainless steel screen-bottom mouse cages for a 14-day test period. Room conditions were maintained at 23–24°C, relative humidity was 40–50%, and a 12-hr light and dark schedule was followed. Feed and water were provided *ad libitum*. Body weights were recorded weekly. Spilled feed was collected on a paper towel under each cage and, at the end of each week after drying at room conditions, was separated from feces using a 16-gauge sieve. The total weight of oven-dry spilled feed was deducted from the weight of the allotted diet corrected to DM basis.

The mice were anesthetized with ether and then decapitated. Plasma was obtained by centrifuging heparinized blood at 1800*g* for 15 min. Two pooled samples of plasma, each from separate groups of three or four mice within each diet group, were deproteinized using 20% trichloroacetic acid in a volume ratio of 1:0.5 (plasma to trichloroacetic acid). The supernatant and the remaining plasma were stored at −18°C until assayed.

Concentrations of AA in deproteinized plasma were measured by ion exchange chromatography using a Perkin Elmer Series 4 Liquid Chromatograph. Plasma urea nitrogen was measured following the release of ammonia from urea by urease and the reaction of ammonia with alkaline hypochlorite and phenol in the presence of sodium nitroprusside (Kit no. 640; Sigma Chemical Co., St. Louis, MO). Total nitrogen in diets was determined by the Kjeldahl method (11).

The design for all experiments was a one-way classification. Observed gains, feed intakes, gain to feed ratios, and urea nitrogen concentrations were analyzed by analysis of variance followed by Waller and Duncan's k ratio test (12) (k ratio = 100) to compare individual means. A k ratio of 100 approximates the 5% level of significance (13). Weight gains adjusted for differences in feed intakes were analyzed using covariance analysis (12). Regression analysis was used to examine effects of increasing dietary nitrogen concentration in each experiment (control data excluded). Most variables showed little response to nitrogen concentration for reasons that are discussed in the text and the regression data were not reported here.

Results

There were no significant increases ($P > 0.05$) in weight gains, adjusted gains, or FE as the dietary nitrogen concentration increased in Experiment 1 (Table VI). For all three variables the performance of mice fed the test diets was inferior to that of the control mice, although the amount of feed eaten was similar for all diets.

In Experiment 2 where true digestible nitrogen and AA concentrations were used in diet formulation, both weight gains and FE decreased ($P < 0.05$) as the nitrogen concentration increased. Intakes of all diets were the same except for Diet 10 which had the highest nitrogen concentration and for which feed intake was significantly reduced ($P < 0.05$). Plasma glycine and urea nitrogen concentrations and phenylalanine to tyrosine (phe to tyr) ratios increased as dietary nitrogen increased. Mice receiving the highest nitrogen concentration were most adversely affected.

Using the same true digestible nitrogen and AA levels as for Experiment 2 but with these nutrients

Table V. Proportions of Nitrogen-Containing Components in the Diet Dry Matter of Experiment 7 (g/kg)

Diet	Total nitrogen by analysis	True digestible nitrogen	Gelatin	Zein	Crystalline amino acids	
					Indispensable	Dispensable
Experiment 7						
31[a]	21.6	19.2	84.8	29.5	13.9	1.0[b]
32	20.6	19.2	—	29.5	36.0	66.9
33	20.9	19.2	84.8	—	22.0	19.9
34[c]	20.6	19.2	—	29.5	36.0	86.5

[a] The same composition as Diet 10, Experiment 2 (Table III) and Diet 25, Experiment 5 (Table IV).
[b] Composition: % dry weight (Diet 31) ala:gly:ser = 25:50:25.
[c] The same as Diet 32 but with glycine replaced by glutamic acid.

Table VI. Weight Gains, Feed Efficiency (Gain/Feed), and Concentrations of Selected Plasma Variables of Mice Fed Test Diets in Experiments 1–3

Diet	Observed gain (g)	Feed intake (DM) (g)	Gain/ feed	Adjusted gain[a] (g)	Plasma[b]				
					Urea nitrogen (mg/dl)	Glycine (μM)	Tyrosine (μM)	Phenylalanine (μM)	Phenylalanine/ tyrosine ratio
Experiment 1									
1	12.1bc[c]	57.9abc	0.208b	12.0c	—	—	—	—	—
2	13.9b	61.6a	0.226b	12.6bc	—	—	—	—	—
3	12.3bc	58.3abc	0.210b	12.2c	—	—	—	—	—
4	13.0bc	56.8bc	0.228b	13.4b	—	—	—	—	—
5	11.7c	53.7c	0.217b	13.1bc	—	—	—	—	—
Control	16.0a	59.0ab	0.272a	15.6a	—	—	—	—	—
SEM[d]	0.70	1.45	0.0091	0.48					
Experiment 2									
6	13.1b	53.8a	0.241b	12.5b	13.5c	226	164	99	0.60
7	13.3b	53.2a	0.249b	13.0b	16.0c	501	122	90	0.74
8	12.1bc	52.6a	0.227bc	12.0b	16.0c	880	104	87	0.84
9	10.4c	52.3a	0.199c	10.4c	19.0b	1124	73	79	1.08
10	7.3d	45.6b	0.150d	9.9c	20.7b	2539	60	78	1.30
Control	16.2a	56.4a	0.287a	14.7a	31.2a	188	173	117	0.68
SEM[d]	0.87	1.99	0.0112	0.44	1.03	—	—	—	—
Experiment 3									
11	11.3b	54.6	0.207c	11.4c	11.2d	241	134	85	0.63
12	12.1b	54.4	0.223bc	12.3b	13.8cd	247	133	84	0.63
13	11.5b	52.9	0.218bc	12.1bc	14.8bc	231	132	79	0.60
14	12.7b	55.5	0.229b	12.5b	17.1b	218	126	77	0.61
15	12.8b	55.3	0.230b	12.6b	17.4b	231	107	75	0.70
Control	16.4a	56.4	0.290a	15.9a	32.7a	161	151	105	0.70
SEM[d]	0.59	1.61	0.0064	0.33	1.07	—	—	—	—

[a] Adjusted for differences in feed intake using covariance analysis.
[b] Plasma amino acid concentrations based on a pooled sample analysis. No statistical comparisons made.
[c] Means followed by different letters are significantly different (k ratio=100).
[d] Standard error of the mean based on 10 observations per treatment group.

provided by a basal diet of constant composition (Experiment 3), the adjusted gains and FE increased slightly relative to those at the lowest level of nitrogen intake and the negative effects of increasing dietary nitrogen on gains and feed intakes were not observed as they had been in the previous experiments (Table VI). Plasma glycine levels and phe to tyr ratios were similar to those of controls.

In these three experiments the weight gains and FE of mice fed the experimental diets were below ($P < 0.05$) those of the control mice fed the AIN diet, although the highest observed or adjusted gains for any experiment were 78–87% of controls.

The results of Experiments 4–6 (Table VII) were similar to those of Experiments 1–3 (Table VI). Most notable were the decreasing gains and FE in Experiment 5 diets based on true digestible nitrogen and AA. These results provided important confirmation of results of Experiments 1–3 despite using a broader array of proteins from both plant and animal sources.

The increasing concentration of gelatin, particularly in diets of Experiments 2 and 5, suggested an association with poor gains and feed efficiency. The highest concentration of glycine in Experiment 5 diets was 21.5 g/kg or about 2% of the diet provided almost entirely by gelatin (Table VIII). The nitrogen provided by this glycine was about 18% of the total dietary nitrogen. By comparison glycine concentration in the casein-based control diet was 3.3 g/kg or approximately 0.3% of the diet.

By replacing gelatin or zein with equivalent amounts of crystalline AA (Diets 32 and 33, respectively) in Experiment 7 (Table IX), the mice did not grow any better compared with those fed the gelatin plus zein diet (Diet 31). In fact, feed intakes were depressed and plasma glycine concentrations tended to rise. However, replacing the glycine from gelatin with glutamic acid (Diet 34) improved FE ($P < 0.05$) and adjusted gains ($P < 0.05$) whereas plasma glycine concentrations were markedly reduced. The phe to tyr ratios were slightly lower with the glutamic acid substitution. The AA composition of gelatin, in particular its high glycine concentration, was primarily responsible for the decreasing gains and FE of mice observed in Experiments 2 and 5.

Discussion

The basal diet that was used for these experimental diets has been used previously (1, 2). Despite some compositional differences between it and the AIN diet

Table VII. Weight Gains, Feed Efficiency (Gain/Feed), and Concentrations of Selected Plasma Variables of Mice Fed Test Diets in Experiments 4–6

Diet	Observed gain (g)	Feed intake (DM) (g)	Gain/ feed	Adjusted gain[a] (g)	Plasma[b]				
					Urea nitrogen (mg/dl)	Glycine (μM)	Tyrosine (μM)	Phenylalanine (μM)	Phenylalanine/ tyrosine ratio
Experiment 4									
16	13.2b[c]	60.6ab	0.217b	12.9b	—	—	—	—	—
17	13.6b	60.8ab	0.223b	13.2b	—	—	—	—	—
18	13.7b	59.5ab	0.229b	13.8b	—	—	—	—	—
19	12.4b	56.6b	0.217b	13.7b	—	—	—	—	—
20	13.5b	58.2b	0.231b	14.1b	—	—	—	—	—
Control	17.7a	63.0a	0.281a	16.4a	—	—	—	—	—
SEM[d]	0.74	1.48	0.0091	0.46	—	—	—	—	—
Experiment 5									
21	12.5bc	53.9ab	0.232bc	12.4b	14.5c	173	163	92	0.56
22	13.7b	56.0ab	0.244b	12.9b	15.0c	388	136	93	0.68
23	11.3cd	52.1ab	0.217c	11.9b	16.4c	655	99	84	0.85
24	10.2d	53.5ab	0.185d	10.2c	20.7b	1062	75	88	1.17
25	7.1c	50.1b	0.141e	8.4d	21.9b	2210	56	73	1.30
Control	16.8a	56.9a	0.295a	15.6a	32.5a	220	215	125	0.58
SEM[d]	0.70	1.59	0.0088	0.39	1.29	—	—	—	—
Experiment 6									
26	13.8b	55.5b	0.248bc	14.0b	13.7e	235	162	95	0.59
27	14.3b	55.7b	0.257bc	14.4b	16.2de	246	150	96	0.64
28	13.6b	55.6b	0.244c	13.7b	16.8cd	257	142	87	0.61
29	14.6b	54.5b	0.268b	15.0b	19.3c	293	133	86	0.65
30	14.3b	54.2b	0.264b	14.8b	22.0b	304	169	113	0.67
Control	17.8a	60.6a	0.294a	16.5a	34.4a	171	179	127	0.71
SEM[d]	0.50	1.19	0.0067	0.37	1.00	—	—	—	—

[a] See footnote *a* to Table VI.
[b] See footnote *b* to Table VI.
[c] See footnote *c* to Table VI.
[d] See footnote *d* to Table VI.

Table VIII. Gelatin and Glycine Contents in the Diet Dry Matter of Experiment 5 (g/kg)

Diet	Total nitrogen by analysis	Gelatin	Glycine from gelatin	Total glycine	Glycine nitrogen[a]
21	14.9	0	0	1.3	0.2 (1.3)
22	16.4	13.7	3.4	4.7	0.9 (5.5)
23	18.0	29.6	7.3	8.5	1.6 (8.9)
24	20.1	55.3	13.6	14.4	2.7 (13.4)
25	22.3	84.8	20.8	21.5	4.0 (17.9)
Control	31.5	0	0	3.3	0.6 (1.9)

[a] Numbers in parentheses show glycine nitrogen as a percentage of total nitrogen.

(8), this basal diet combined with the protein source of the AIN diet (20% casein + 0.3% DL-methionine) resulted in weight gains in weanling mice equal to those fed the AIN diet (unpublished data). The inferior growth and FE observed in this study were probably due to the DAA fraction since the IAA were provided at the requirement levels (1, 2).

Glycine was the DAA that varied most widely in dietary concentration. The glycine content of gelatin is about 24% (10) and diets with the highest gelatin level contained 2.15% glycine, equivalent to 18% of the dietary nitrogen. Although a reduction in weight gains was not observed in rats fed AA diets containing up to 3% glycine (14), some growth reduction usually occurs at higher levels of glycine. An AA-based diet containing 4.25% glycine caused lower gains and FE in rats (6). Similar growth inhibition in rats has been reported when glycine supplied a large proportion of the nonspecific nitrogen (3, 5, 15). In contrast, the reduced growth of chicks fed an AA- or protein-supplemented gelatin diet with a high glycine concentration was believed to be due to a methionine deficiency rather than to an excess of glycine (7). For chicks the requirements for both methionine and glycine are relatively large (16) which might partly explain the difference.

Glycine has been found to be anorexigenic in rats, apparently due to ammonia yielded by the glycine (17). Sauberlich (5) found no reduction in feed intakes in rats fed a diet containing 5% glycine but weight gains were lower which resulted in lower FE. Feed intakes in the current study declined only slightly in Experiments 2 and 5 and FE was reduced. Weight gains adjusted for differences in feed intake were also reduced; therefore,

Table IX. Weight Gains, Feed Efficiency (Gain/Feed), and Concentrations of Selected Plasma Variables in Mice Fed Test Diets in Experiment 7

Diet	Observed gain (g)	Feed intake (DM) (g)	Gain/ feed	Adjusted gain[a] (g)	Plasma[b]				
					Urea nitrogen (mg/dl)	Glycine (μM)	Tyrosine (μM)	Phenylalanine (μM)	Phenylalanine/ tyrosine ratio
Experiment 7									
31	12.8bc[c]	59.4ab	0.215c	12.4c	27.0b	1952	78	77	0.99
32	12.2c	55.1c	0.221c	12.7c	25.4b	2335	73	59	0.81
33	11.8c	55.4c	0.212c	12.3c	25.3b	2212	78	66	0.85
34	13.5b	56.9bc	0.238b	13.7b	28.0b	575	89	69	0.78
Control	17.6a	61.7a	0.285a	16.7a	37.6a	276	170	95	0.56
SEM[d]	0.44	1.19	0.0064	0.36	1.53	—	—	—	—

[a] See footnote *a* to Table VI.
[b] See footnote *b* to Table VI.
[c] See footnote *c* to Table VI.
[d] Standard error of the mean based on 12 observations per treatment group.

it appears that appetite depression was not important in explaining the effect of glycine.

The mechanism by which glycine affects growth and metabolism in mice may relate to its ammonia-generating potential. Dogs infused intravenously with glycine showed increased blood ammonia levels which were related to the amount of glycine infused (18). Infusion of a casein hydrolyzate providing amino nitrogen at the same rate resulted in a much smaller increase in blood ammonia level but a greater increase in blood urea. Glycine given orally or intravenously to human subjects with liver disease is one of the most ammonigenic AA (4). Glycine is rapidly deaminated in the liver and ammonia formation is an important step in the entry of glycine nitrogen into the urea cycle (19).

Changes in the phe to tyr ratio similar to those seen here have been observed in rats (20–22) and seem to be associated with a pathologic state. Elevated plasma concentrations of phenylalanine and methionine are found in patients with liver disease (23, 24), indicating that there is delayed conversion to the DAA and reduced clearance of the two IAA. In cirrhotic patients high plasma phenylalanine concentrations were associated with impaired ammonia tolerance (25). In the mice in the present investigation, plasma phenylalanine did not increase but tyrosine dropped markedly, leading to high phe to tyr ratios. The relationship of the phe to tyr ratio to ammonia concentration is uncertain because ammonia was not measured, but there is a strong possibility that the mice fed high glycine diets were subject to a persistent ammonia load.

In the present experiments arginine was considered a DAA (2). Although an increase in the ammonia load can increase the need for arginine (26), there was about four times as much arginine in gelatin (8.59%) (10) as in casein (2.32%) and the highest level of gelatin in the diet (8.48%) provided 0.79% arginine. Unless the need for arginine was unusually high, a shortage of this AA was not a likely explanation for the poor performance of mice fed high gelatin (high glycine) diets.

The possibility exists that an IAA was limiting in the experimental diets. If such a deficiency existed, it was of a relatively mild degree since the fastest growing test group overall averaged about 80% of the gains seen with the AIN control diet. Under these circumstances a dietary excess of most DAA would result in greater urea excretion and decreased efficiency of nitrogen utilization but not growth impairment or other evidence of toxicity, both of which were observed in Experiments 2 and 5, in response to increased dietary levels of glycine.

These experiments provided evidence for differences in the ability of purified proteins to provide AA nutrition for mice even though the IAA were supplied at recommended levels. These experiments identified glycine as the AA in gelatin that interfered with nitrogen utilization. For mice and probably for other simple-stomached animals, gelatin as the principal source of dietary nitrogen poses a risk to the metabolism of the animal.

The assistance of B. Chatfield, K. Johns, S. McCormac, L. Atamanenko, and S. Hodgson in carrying out this series of experiments and in manuscript preparation is gratefully acknowledged. Funding was provided by the Natural Sciences and Engineering Research Council of Canada, Ottawa.

1. John A-M, Bell JM. Amino acid requirements of the growing mouse. J Nutr **106**:1361–1367, 1976.
2. Bell JM, John A-M. Amino acid requirements of growing mice: Arginine, lysine, tryptophan and phenylalanine. J Nutr **111**:525–530, 1981.
3. Rechcigl M Jr, Loosli JK, Williams HH. The net utilization of nonspecific nitrogen sources for the synthesis of non-essential amino acids. J Nutr **63**:177–192, 1957.
4. Rudman D, Galambos JT, Smith RB III, Salam AA, Warren WD. Comparison of the effect of various amino acids upon the

blood ammonia concentration of patients with liver disease. Am J Clin Nutr **269**:916–925, 1973.
5. Sauberlich HE. Studies on the toxicity and antagonism of amino acids for weanling rats. J Nutr **75**:61–72, 1961.
6. Hepburn FN, Bradley WB. Effect of glutamine on inhibition of rat growth by glycine and serine. J Nutr **94**:504–510, 1968.
7. Ashley JH, Fisher H. The amino acid deficiencies of gelatin for the growing chicken. Poult Sci **45**:541–546, 1966.
8. American Institute of Nutrition. Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. J Nutr **107**:1340–1348, 1977.
9. National Research Council. Nutrient Requirements of Laboratory Animals. No. 10, 3rd rev. ed. Washington, DC: National Academy of Sciences-National Research Council, 1978.
10. Keith MO, Bell JM. Digestibility of nitrogen and amino acids in selected protein sources fed to mice. J Nutr **118**:561–568, 1988.
11. Association of Official Analytical Chemists. Official Methods of Analysis. 13th ed. Washington, DC: AOAC, 1980.
12. Statistical Analysis System. SAS User's Guide: Statistics. Gary, NC: SAS Institute Inc., 1982.
13. Steel RGD, Torrie JH. Principles and Procedures of Statistics. A Biometrical Approach. New York: McGraw-Hill, 1980.
14. Rogers QR, Chen DM-Y, Harper AE. The importance of dispensable amino acids for maximal growth in the rat. Proc Soc Exp Biol Med **134**:517–522, 1970.
15. Harper AE, Benevenga NJ. Effects of disproportionate amounts of amino acids. In: Lawrie RA, Ed. Proteins as Human Food. Westport, CT: AVI Publishing Co. Inc., p417–447, 1970.
16. National Research Council. Nutrient Requirements of Poultry. 7th rev. ed. Washington, DC: National Academy of Sciences-National Research Council, 1977.
17. Russek M. Hepatic receptors and the neurophysiological mechanisms controlling feeding behaviour. Neurosci Res **4**:214–282, 1971.
18. Harper HA, Najarian JS, Silen W. Effect of intravenously administered amino acids on blood ammonia. Proc Soc Exp Biol Med **92**:558–560, 1956.
19. White A, Handler P, Smith LE. Principles of Biochemistry. 5th ed. New York: McGraw-Hill, 1973.
20. Keith MO, Tryphonas L. Choline deficiency and the reversibility of renal lesions in rats. J Nutr **108**:434–446, 1978.
21. Wannemacher RW Jr, Dinterman RE, Powanda MC. Phenylalanine metabolism in the infected rat. Fed Proc **34**:900, 1975.
22. Wang M, Vyhmeister I, Kopple JD, Swendseid ME. Effect of protein intake on weight gain and plasma amino acid levels in uremic rats. Am J Physiol **230**:1455–1459, 1976.
23. Fischer JE, Yoshimura N, Aquirre A, James JH, Cummings MG, Abel RM, Deindorfer F. Plasma amino acids in patients with hepatic encephalopathy. Am J Surg **127**:40–47, 1974.
24. Iob V, Coon WW, Sloan M. Altered clearance of free amino acids from plasma of patients with cirrhosis of the liver. J Surg Res **6**:233–239, 1966.
25. Levine RJ, Conn HO. Tyrosine metabolism in patients with liver disease. J Clin Invest **46**:2012–2020, 1967.
26. Du Ruisseau JP, Greenstein JP, Winitz M, Birnbaum SM. Studies on the metabolism of amino acids and related compounds *in vivo*. IV. Blood ammonia and urea levels following intraperitoneal administration of amino acids and ammonium acetate, and the effect of arginine thereon. Arch Biochem Biophys **64**:355–367, 1956.