The Effects of Dietary Amino Acid Levels upon Lysine and Methionine Requirements for Growing Rats

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Summary The amino acid requirement has been investigated by many laboratories since 1931. Authors considered that the requirement was influenced by the protein level of the basal diet and also by the experimental method. In light of those earlier studies, the present study investigated the effect on lysine and methionine requirements by modifying total nitrogen level or essential amino acid level. The result of our study confirmed that when the amino acid requirement for maximum growth was expressed as the dietary percentage, amino acid to total amino acid ratio, and amino acid to total nitrogen ratio, the requirement had changed according to not only essential amino acids but also to non-essential amino acids. Furthermore, we investigated the relationship between amino acid intake and body protein gain, and found that 6.2 mg lysine and 9.0 mg methionine were required for the maintenance of a rat weighing 80 g and 71.6 mg of lysine and 48.8 mg of methionine were required for one gram of body protein gain. The lysine and methionine intakes required for one gram of protein gain were equivalent to 100% and 124% of amino acid contents of body protein, respectively.

Key Words lysine requirement, methionine requirement, E/TN ratio, dietary N level, maximum growth, body protein gain

The essential amino acid requirement for growing rats has been investigated by many researchers since Rose (1). For instance, if we show the value of the lysine requirement estimated as a percentage of diet, we get, according to Rose 1.0% (1), Rama Rao 0.9% (2), Miyazaki 0.46% (3) and Yoshida 0.3% (4), etc. If the requirements are expressed in the Lys/Thr ratio, we get; according to Rose 2.0 (1), Rama Rao 1.8 (2), Miyazaki 1.3 (3), and Yoshida 1.5 (4). We attribute the discrepancies in the contrasting sets of figures to the different methods of experiment. The experimental condition will be affected by the strain, experimental period, amino acid level and composition of diet.

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Generally, the dietary source of nitrogen is the predominant factor in estimation of the requirement. The dietary amino acid levels in experiments of the amino acid requirement have not been constant—e.g., Yoshida 5% (4), Rama Rao 10% (2) and Rose 12.5% (1). However, according to Grau (5) and Bressani (6), the lysine requirement (as a % of diet) increases with the protein level.

Previously, we confirmed the growth of rats fed 5%, 10%, 15%, and 20% levels using the whole egg pattern amino acid mixture; 10% was identified as the optimum level of whole egg pattern amino acid mixture at which maximum growth is attained. It was thus necessary to provide the 5% and 10% levels for determining the relationships between dietary nitrogen level and amino acid requirement. It is known as relationship of minimum amino acid requirement expressed as a percentage of the diet to protein content of the diet in rats. Although the test with lysine was reported in the literature (6), methionine experiment has not been previously reported. Our laboratory has been conducting studies involving experiment with methionine.

There was no literature on the relationship of E/N ratio in amino acid mixture to minimum amino acid requirement of rat. A similar observation was made by Young and Zamora (13), Stucki and Harper (14) and Matsuno et al. (15) in tests with body weight gain versus E/N ratio in amino acid mixture, but there was no literature on the relationship of E/N ratio in amino acid mixture to minimum amino acid requirement in rats. The following investigation extends our previous work regarding the amino acid requirement.

There are certain problems with the criteria for estimating the requirement or expression of requirement. For instance, the amino acid requirement for growing rats has generally been expressed with the minimum dietary percentage for the maximum weight gain. Other problems include the amino acid requirement with the nitrogen balance method, the plasma amino acid level, the amino acid catabolism, and the like. In this study, we investigated the relationship between the body protein gain and the amino acid intake excluding weight gain. Furthermore, we investigated the expression of the amino acid requirement. That is to say, the requirements were expressed in units per one gram of body protein gain, minimum dietary percentage for maximum growth, and the ratio for essential amino acids or total amino acids. The requirement values were altered in various ways.

The purpose of the present study was to investigate the relationship between the dietary amino acid level and the body weight gain, expressing the amino acid requirement, and to thus resolve and clarify problems of amino acid requirement. The final purpose of this further study was to determine amino acid requirement for growing rats.

MATERIALS AND METHODS

Experiment 1

We selected male Wistar strain rats, weighing approximately 60 g for the
experiment. Rats received a 20% casein diet, supplemented with 0.3% DL-methionine. When rats attained a weight of 80 g, the diet was changed to the experimental diet.

The animals were housed in individual cages with a controlled temperature of 23 ± 1°C and a 12 h light-dark cycle. The water and diet were fed ad libitum. The food intake and body weight were recorded every day during the experimental period.

The quantitative relationship between the amino acid level and weight gain was studied to facilitate the selection of amino acid for Experiment 2. Amino acid mixtures in diets, whose compositions simulated those of whole egg protein, were adjusted by nitrogen content multiplied by 6.25. In the first experiment, the body weight change was determined in growing rats fed on the 0%, 5%, 10%, 15%, and 20% amino acid diets during the 21-day experimental period.

Experiment 2

The experimental conditions were identical to those in Experiment 1 except that the experimental period was 14 days.

We used entirely L-amino acid mixture as nitrogen source of the basal diet. The experimental diets contained essential and nonessential amino acid mixture, and the compositions simulated those of whole egg protein as shown in Table 2. The proportion of essential amino acids in the amino acid mixture and the content of amino acid mixture in diets were adjusted by the nitrogen content.

As shown in Table 1, there were four experimental groups in the experiment of lysine requirement and three in the experiment of methionine requirement; the groups received different proportions of essential amino acids to total amino acids or nitrogen contents of total amino acids. The experimental diets of group A contained amino acid mixtures at the 10% (N x 6.25) level in total, while those of group B contained amino acid mixtures at the 5% (N x 6.25) level in total. The experimental diets of group C contained amino acid mixtures at the 10% (N x 6.25) level and the essential amino acids were the same level as group B. The experimental diets of group D contained amino acid mixtures at the 0% (N x 6.25) level, and the essential amino acids were the same level as group B.

| Group | Total N | EAA N | Lys | Met |
|-------|---------|-------|-----|-----|
| A     | 1.6     | 1.0   | 0, 0.1, 0.2, 0.4, 0.55, 0.7, 0.85, 1.0 |     |
| B     | 0.8     | 0.5   | 0, 0.1, 0.2, 0.39, 0.5, 0.55, 0.6, 0.67, 1.0 |     |
| C     | 1.6     | 0.5   | 0, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.6 |     |
| D     | 1.0     | 1.0   | 0, 0.1, 0.2, 0.3, 0.35, 0.4, 0.45, 0.6 | 0, 0.1, 0.2, 0.8 |

Table 1. Amino acid levels of experimental diets (% in diet).
Table 2. Composition of amino acid mixture.

|           | Without lysine (mg/Ng) | %   | Without methionine (mg/Ng) | %   |
|-----------|------------------------|-----|----------------------------|-----|
| **EAA**   |                        |     |                            |     |
| l-Ile     | 866                    | 11.7| 786                        | 11.1|
| l-Leu     | 1,214                  | 16.3| 1,102                      | 15.6|
| l-Lys·HCl | 0                      | 0   | 1,090                      | 15.4|
| l-Met     | 463                    | 6.3 | 0                          | 0   |
| l-Cys     | 335                    | 4.5 | 0                          | 0   |
| l-Phe     | 789                    | 10.7| 716                        | 10.1|
| l-Tyr     | 573                    | 7.8 | 520                        | 7.4 |
| l-Thr     | 705                    | 9.6 | 640                        | 9.1 |
| l-Trp     | 205                    | 2.8 | 186                        | 2.6 |
| l-Val     | 943                    | 12.8| 856                        | 12.1|
| l-Arg     | 839                    | 11.4| 762                        | 10.8|
| l-His·HCl·H₂O | 452               | 6.1 | 410                        | 5.8 |
| **Total** | 7,384                  | 100.0| 7,068                     | 100.0|
|           |                        |     |                            |     |
| **NEAA**  |                        |     |                            |     |
| l-Ala     | 1,117                  | 13.6| 1,117                      | 13.6|
| l-Asp     | 1,815                  | 22.2| 1,815                      | 22.2|
| l-Glu     | 2,403                  | 29.4| 2,403                      | 29.4|
| l-Gly     | 625                    | 7.6 | 625                        | 7.6 |
| l-Pro     | 785                    | 9.6 | 785                        | 9.6 |
| l-Ser     | 1,443                  | 17.6| 1,443                      | 17.6|
| **Total** | 8,188                  | 100.0| 8,188                     | 100.0|

diets of group D contained the same essential amino acids as group A but without nonessential amino acids.

The levels of the lysine and methionine in each group are shown in Table 1. With reference to the whole egg pattern (7) and the amino acid requirement pattern which has been reported (8), the additional amino acid level was altered from 0% to the level of the plateau in the body weight. The content of nitrogen in the meal was kept constant by adjusting the amount of the nonessential amino acids as shown in Table 2, depending on the additional amounts of limiting amino acids added to the diet.

In addition, oil, vitamins, minerals, and cellulose were supplied as listed in Table 3. The experimental diet was maintained as an isocaloric type by controlling the carbohydrate content (α-starch and sucrose, 2:1 mixture) according to the amount of total amino acid in the diets.

The food intake was measured in a unit of 10 mg using an electronic reading balance (SARTORIUS, West Germany) for determining the amino acid intakes exactly. And these leavings of amino acid diets were collected exactly.

To determine the composition of the rat carcass, it was analyzed as follows.
Table 3. Composition of an experimental diet (g/kg).

| Component                  | Amount (g/kg) |
|----------------------------|---------------|
| Lys·HCl                    | 6.8           |
| Lys(--)-EAA mixture        | 61.7          |
| NEAA mixture               | 53.5          |
| α-Starch                   | 499           |
| Sucrose                    | 249           |
| Corn oil                   | 50            |
| Vitamin mixture            | 10            |
| Mineral mixture            | 50            |
| Cellulose                  | 20            |
| Total                      | 1,000         |

*a* Lysine-deficient essential amino acid mixture of whole egg pattern (Table 2). *b* Refer to Table 2. *c* Supplied as the following (per kilogram of diet): retinyl acetate, 5,000 IU; cholecalciferol, 1,000 IU; tocopheryl acetate, 50 mg; menadione, 52 mg; thiamin·HCl, 12 mg; riboflavin, 40 mg; pyridoxine·HCl, 8 mg; cyanocobalamin, 0.005 mg; ascorbic acid, 300 mg; d-biotin, 0.2 mg; folic acid, 2 mg; calcium pantothenate, 50 mg; p-aminobenzoic acid, 50 mg; nicotinic acid, 60 mg; inositol, 60 mg; choline chloride, 2,000 mg.

d Supplied as the following (milligrams per kilogram of diet): CaHPO₄·H₂O, 7,280; KH₂PO₄, 12,860; NaH₂PO₄·H₂O, 4,675; NaCl, 2,330; Ca-lactate, 17,545; Fe-citrate, 1,590; MgSO₄, 3,585; ZnCO₃, 55; MnSO₄·4·H₂O, 60; CuSO₄·5H₂O, 15; KI, 5.

Frozen carcasses of rats were sliced into pieces 5 mm thick and dried in an oven at 95°C for 48 h. The body water was calculated as the difference between the wet and dry carcass weights. The nitrogen content of the carcass was analyzed by the semimicro Kjeldahl method (9), and the protein content was calculated as N×6.25.

Most estimates of requirements were based upon the minimum dietary percentage for the maximum weight gain. In this paper, the methods of estimating the amino acid requirement were as follows. A straight line was drawn along the points which fell below the maximum response and the requirement was taken at the intersection of this line with a line drawn at the maximum response. A line drawn at the maximum response was the average weight gain of a group which showed the highest growth rate and other groups which showed no statistically significant difference from the highest group by the Student’s *t*-test (10).

The requirement for a unit gain in body protein is determined as follows. The linear regression of body protein gain (g/day) on each essential amino acid intake (mg/day) was obtained by taking the intake range below maximum body weight gain. The slope of this regression line was taken as the requirement figure of amino acid for one gram gain of body protein.

**RESULTS**

*Experiment 1*

For growing rats, the body weight gain (g/day) was $-1.19 \pm 0.18$, $3.14 \pm 0.80$. 
Fig. 1. Body weight gain (g/day) of rats given l-amino acid diets with whole egg pattern containing varying amino acid levels. Vertical lines represent plus or minus one standard deviation for each group of 6 rats.

Fig. 2. Change in food intake in relation to dietary lysine contents. Experimental group A (■), group B (△), group C (○) and group D (●); further details are given in Table 1.

6.12 ± 0.61, 5.07 ± 0.34, and 4.87 ± 0.65 in the 0%, 5%, 10%, 15%, and 20% amino acid levels, respectively, as shown in Fig. 1. Since these values showed the maximum body weight gain of the whole egg pattern, approximately 6 g per day was obtained at the 10% amino acid diet.

**Experiment 2**

1. **Lysine requirement.** The results of food intake is shown in Fig. 2. The food intake was about 6 g/day in the lysine-deficient diet. But the food intake was 15.20 ± 0.68 g/day in the group A diet to which 0.55% lysine had been added as a salient feature.
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Fig. 3. Change in body weight in relation to dietary lysine contents. Weight gain of rats given L-amino acid diets containing either 1.6% (■) or 0.8% (△) dietary N and varying in concentration of total essential amino acid N, 0.5% (○) and 1.0% (●).
The T1.6 and E0.5 represent 1.6% total N and 0.5% essential amino acid N in diet.声道 whole egg pattern level; 时间节点, calculated requirement value.

The change in body weight in relation to dietary amino acid content of groups A, B, C, and D is shown in Fig. 3. The maximum weight gain was different from the total dietary amino acid or essential amino acid level. It was confirmed that the weight gain in each group significantly increase as lysine was added to get the maximum weight gain. The highest level of the maximum weight gain of 10% amino acid diet was 5.96 ± 0.45 g/day (group A), but the maximum weight gain of group C was significantly (p < 0.001) decreased by 4.07 ± 0.60 g/day as the essential amino acid level was reduced by one half. The maximum weight gain in group B was significantly (p < 0.001) decreased by 2.32 ± 0.58 g/day as the total amino acid level was reduced by one half. A new, maximum growth of group D which used essential amino acid as dietary nitrogen source, which was 1.16 ± 0.33 g/day, was inferior in growth to the 5% amino acid diet (group B).

The maximum growth was different for each group in the dietary amino acid level, but the change in body weight in relation to dietary lysine content between 0% to the level which fell below the maximum response was a linear line independent of dietary amino acid level and dietary essential amino acid level. This relation was estimated in a regression equation as follows: \( Y = 12.60X - 1.01 \) \( (r = 0.96, n = 88) \), where \( Y \) represents body weight gain (g/day) and \( X \) is the lysine % in diet.

The minimum percentage of dietary lysine required for maximum growth was defined as the intersection of the regression line estimated by taking the relationship between lysine % in the diet and weight gain: \( Y = 12.60X - 1.01 \), with a line drawn at the maximum growth \( Y = 5.96, Y = 2.32, Y = 4.07, \) and \( Y = 1.16 \) in groups A, B, C,
Table 4. Requirement for maximum growth.

| Group | % of diet | A/E   | A/TN  | Maximum growth (g/day) |
|-------|-----------|-------|-------|------------------------|
| Lysine requirement |
| A     | 0.553     | 0.083 | 0.346 | 5.96 ± 0.45 (n = 20)   |
| B     | 0.264     | 0.080 | 0.330 | 2.32 ± 0.58 (n = 23)   |
| C     | 0.403     | 0.117 | 0.252 | 4.07 ± 0.60 (n = 15)   |
| D     | 0.172     | 0.028 | 0.201 | 1.16 ± 0.33 (n = 10)   |

| Methionine requirement |
| A     | 0.486     | 0.073 | 0.304 | 5.46 ± 0.57 (n = 24)   |
| B     | 0.270     | 0.080 | 0.337 | 2.17 ± 0.56 (n = 27)   |
| C     | 0.420     | 0.119 | 0.262 | 4.46 ± 0.77 (n = 17)   |

and D, respectively).

Some of the estimated values are shown in Table 4. By reducing the amino acid level in the basal diet by half, for example 0.553% in 10% amino acid diet (group A) and 0.264% in 5% amino acid diet (group B), the value was also reduced by half. The essential amino acid level of an experimental diet was identical with group B and the total nitrogen level of an experimental diet was identical with the 10% amino acid diet, and the value of the requirement increased to 0.403% in group C. Group D’s amino acid diet without nonessential amino acid was 0.172% regardless of the essential amino acids level being identical with group A. The lysine requirements of dietary percentage in this study were equal to or lower than the lysine content of the whole egg pattern irrespective of dietary amino acid level.

In general, the amino acid requirement, expressed as a percentage of the diet, tends to increase as protein content increases but may remain constant or decrease slightly when expressed as A/TN ratio. Accordingly, the requirement for an amino acid expressed as the ratio of lysine to essential amino acids (A/E) or the ratio of lysine to the total nitrogen (A/TN) resulted in the following. A/E was 0.028 in group D which used all essential amino acids, and was less than 0.083 in group A. Group A was identical with group B in A/TN and A/E requirement. But, A/E of group C was the highest compared with the other groups.

For the purpose of estimating the requirement for maintenance of body protein or one gram of body protein gain, we studied the relationship between body protein gain (g/day) and lysine intake (mg/day). The change in body protein in relation to lysine intake (mg/day) is illustrated in Fig. 4. In every one of the amino acid levels, the response of body protein (mg/day) in relation to lysine intake (mg/day) was a linear line between zero intake to a level which fell below the maximum response. A significant difference was calculated between the regression equations by the statistical analysis using the F-test on variance and t-tests on slope and Y-intercept (11) from group A with 10% amino acid level. The regression lines were drawn as follows: The regression equation for group A with 10% amino acid level

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Fig. 4. The relationship between the change in body protein and lysine intakes. Experimental group A (■), group B (△), group C (○), and group D (●); further details are given in Table 1.

was $Y = 0.0130X - 0.0684$ ($n=25$, $r=0.9941$), where $X =$ lysine intake (mg/day) and $Y =$ body protein gain (g/day). The points adapted in calculation were 0%, 0.1%, 0.2%, 0.4%, and 0.55% that ranged from 0% to maximum growth. The regression equation for group B with 5% amino acid level was $Y = 0.0134X - 0.0993$ ($n=29$, $r=0.9708$, no significant difference in variance, no significant difference in $Y$-intercept, no significant difference in slope). The points adapted in calculation were 0%, 0.2%, 0.25%, and 0.3% that range from 0% to maximum growth. The regression equation for group C with a half essential amino acid level and 10% amino acid level was $Y = 0.0149X - 0.0906$ ($n=29$, $r=0.9797$, no significant difference in variance, no significant difference in $Y$-intercept, no significant difference in slope). The points adapted in calculation were 0%, 0.2%, 0.25%, 0.3%, 0.35%, and 0.4% that ranged from 0% to maximum growth. And the regression equation of group D with solely essential amino acid was $Y = 0.0152X - 0.0808$ ($n=15$, $r=0.9694$, no significant difference in variance, no significant difference in $Y$-intercept, no significant difference in slope). The points adapted in calculation were 0%, 0.1%, and 0.2% that ranged from 0% to maximum growth. For the above equations, the relationships between lysine intake (mg/day) and body protein gain (g/day) were independent of the basal amino acid levels. Then the interrelation was calculated expressing the conclusions from groups A, B, C, and D in the following equation: $Y = 0.0136X - 0.0758$ ($n=88$, $r=0.98$) where $Y$ represents body protein gain (g/day) and $X$ is lysine intake (mg/day).

For the purpose of estimating lysine intake for one gram of body protein gain,
we estimated the reverse regression equation; thus, \( Y = 71.6X + 6.2 \) \((n = 88, r = 0.98)\) where \( Y \) represents lysine intake (mg/day) and \( X \) is body protein gain (g/day).

The conclusion is that 6.2 mg of lysine was required for maintenance of a rat weighing 80 g and 71.6 mg of lysine was required to gain one gram of body protein.

2. Methionine requirement. The results of food intake are shown in Fig. 5. Three g/day of food intake in the methionine-deficient diet was lower than \( 6.27 \pm 0.54 \) g/day in the protein-free diet. Conversely, \( 14.89 \pm 1.07 \) g/day \((n = 6)\) of food intake in the 0.45% methionine diet of group C was the highest. The food intake in a 0% methionine diet was \( 3.37 \pm 0.34 \) g/day in group A which was significantly \((p < 0.001)\) lower than \( 4.46 \pm 0.30 \) g/day in group B. Also the food intake of group A was significantly \((p < 0.005)\) lower than \( 4.08 \pm 0.48 \) g/day in group C. There was no significant difference between the food intake of the protein-free diet and the food intake of the 0% lysine diet, but the food intake of the 0% methionine diet was significantly \((p < 0.001)\) lower than the protein-free diet.

The change in body weight in relation to dietary amino acid content of group A, B and C is shown in Fig. 6. The dose-response regression equation was estimated from this relationship; thus: \( Y = 15.23X - 1.94 \) \((n = 95, r = 0.96)\) where \( Y \) represents body weight gain (g/day) and \( X \) is methionine % in diet. The maximum weight gain was significantly \((p < 0.001)\) different from the total dietary amino acids and essential amino acids levels. The maximum growth was \( 5.46 \pm 0.57 \) g/day with the 10% amino acid diet (group A) and \( 2.17 \pm 0.56 \) g/day with 5% amino acid diet (group B). When the essential amino acid level was identical with the 5% amino acid diet and total nitrogen level was identical with the 10% amino acid diet, the maximum growth was \( 4.46 \pm 0.77 \) g/day (group C).

![Fig. 5. Change in food intake in relation to dietary methionine contents. Experimental group A (■), group B (△), and group C (○); further details are given in Table 1.](image-url)
Fig. 6. Change in body weight in relation to dietary methionine contents. Weight gain of rats given L-amino acid containing either 1.6% (●) or 0.8% (△) dietary N and varying in concentration of total essential amino acid (○). The T1.6 and E0.5 represent 1.6% total N and 0.5% essential amino acid N in diet.

The minimum dietary percentage for maximum growth could be obtained by putting maximum growth in each group in the regression equation of $Y=15.23X-1.94$. As illustrated in Table 4, the results of amino acid on the basis of the whole egg pattern were 0.486% of 10% amino acid level (group A) and 0.270% of 5% amino acid level (group B). The value of group C was 0.420% which was similar to the value of the 10% amino acid diet (group A).

Furthermore, the value was expressed as the ratio of methionine to essential amino acids (A/E) and the ratio of methionine to the total nitrogen (A/TN). In the case of group A and group B, where the essential amino acid level and nonessential amino acid level were identical with each other, the A/E values were nearly 0.073 and 0.080, respectively. Also, the 0.304 of group A and the 0.337 of group B in A/TN showed a resemblance. Group C which was fed less essential amino acid was 0.119 in A/E, which was higher than the other 2 groups, but was reversely lower than the other 2 groups when expressed as A/TN.

The change in body protein gain in relation to methionine intake is illustrated in Fig. 7. The body protein gain was directly proportional to methionine intake in the three experiments from 0% to the level which fell below the maximum response. The regression equation of body protein gain (g/day) on each essential amino acid intake (mg/day) was obtained by taking the intake range below maximum growth. A significant difference was calculated between the regression equations by the statistical analysis using F-test on variance and t-tests on slope and Y-intercept ($H_0$) from group A with the 10% amino acid diet. The regression equations were drawn as follows: The regression equation for group A with the 10% amino acid diet was...
Fig. 7. The relationship between the change in body protein and methionine intakes. Experimental group A (■), group B (△), and group C (○); further details are given in Table 1.

\[ Y = 0.0193X - 0.1533 \quad (n=30, \ r=0.9827) \]

where \( X \) = methionine intake (mg/day) and \( Y \) = body protein gain (g/day). The points adapted in calculation were 0%, 0.1%, 0.2%, 0.3868%, and 0.5% that ranged from 0% to maximum growth. The regression equation for group B with the 5% amino acid diet was

\[ Y = 0.0179X - 0.1651 \quad (n=30, \ r=0.9460, \ no \ significant \ difference \ in \ variance, \ no \ significant \ difference \ in \ Y\text{-intercept}, \ no \ significant \ difference \ in \ slope) \]

and the regression equation for group C was

\[ Y = 0.0209X - 0.1445 \quad (n=30, \ r=0.9874, \ no \ significant \ difference \ in \ variance, \ no \ significant \ difference \ in \ Y\text{-intercept}, \ no \ significant \ difference \ in \ slope) \]

The points of group B adapted in calculation were 0%, 0.1%, 0.15%, 0.2%, 0.25%, and 0.3%. The points of group C adapted in calculation were 0%, 0.1%, 0.2%, 0.3%, and 0.35%. In the above equations, the relationship between methionine intake (mg/day) and body protein gain (g/day) was independent of the dietary amino acid levels. Then the interrelation was calculated bringing together the results of groups A, B, and C into the following equation:

\[ Y = 0.0193X - 0.1609 \quad (n=95, \ r=0.97) \]

where \( Y \) represents body protein gain (g/day) and \( X \) is methionine intake (mg/day). For the purpose of estimating the methionine intakes for one gram of body protein gain, we estimated the reverse regression equation from the interrelationships; thus:

\[ Y = 48.8X + 9.0 \quad (n=95, \ r=0.97) \]

where \( Y \) represents methionine intake (mg/day) and \( X \) is body protein gain (g/day). The conclusion is that 9.0 mg of methionine was required for maintenance of rats weighing 80 g and 48.8 mg of methionine was required for one gram of body protein gain.

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DISCUSSION

The estimation of the amino acid requirement from the relationship between the dietary amino acid percentage and weight gain is a simple and common method. Bressani and Mertz (6) pointed out that lysine requirements increased to 0.84% from 0.54% when dietary protein levels increased to 16% from 8%. The present findings confirmed previous observations that lysine requirements increased to 0.55% from 0.26% when dietary protein levels increased to 10% from 5%. Leveille et al. (12) reported that the sulfur amino acid requirements for growth of mice fed 1.5% and 2.5% levels of nitrogen were 0.20% and 0.53%, respectively. The results of our experiment using rats were 0.486% of 10% amino acid diets (group A) and 0.270% of 5% amino acid diets (group B). In this manner when the requirement was expressed as dietary percentage, the requirements were very different.

On the other hand, Bressani's study showed that the lysine requirements calculated as a ratio of lysine to the total nitrogen (A/TN) were 0.42, 0.35, and 0.32 when rats were fed diets containing 8%, 12%, and 16% protein. The results of our experiment were 0.346 with the 10% amino acid diets (group A) and 0.330 with the 5% amino acid diet (group B) in A/TN. The methionine requirements calculated as a ratio of methionine to the total nitrogen (A/TN) were 0.337 and 0.304 when rats were fed diets containing 5% and 10% amino acids, respectively. When the amino acid requirement for maximum growth was calculated as a ratio of amino acid to the total amino acid, the requirements should be regarded as an approximation.

In the present finding, the maximum growth of whole egg pattern amino acid mixture was obtained at the 10% level. The 10% amino acids was regarded as the control level and we investigated the effects upon the amino acid requirement for growing rats of dietary nitrogen levels using 5% amino acid level, setting limits to a half.

The effects on growth of altering the proportions of essential to nonessential amino acids are well known since a report by Young and Zamora (13), Stucki and Harper (14) and Matsuno et al. (15). Young and Zamora (13) reported that for the 2.23% dietary nitrogen series, maximum growth was achieved when the ratio of total essential amino acids per gram total dietary nitrogen (E/TN ratio) was between 3.37 and 4.71, and at the highest essential amino acid intake, growth rate declined. The ratio of total essential amino acids per gram total dietary nitrogen (E/TN ratio) was 4.30, 4.30, 2.15, and 7.18 for groups A, B, C and D, respectively. The authors concur with Young and Zamora (13) that maximum weight gain of 4.30 of E/TN ratio in group A was the highest and maximum weight gain of solely essential amino acid diet was the lowest as compared with other groups. When the lysine requirement of group C was expressed as a dietary percentage and A/E ratio, the requirements were higher than that of group B. The dietary essential amino acid level of group C was equal to that of group B, but dietary nonessential amino acid level of group C was threefold that of group B. Thus the minimum dietary methionine percentage for maximum growth increased along with rising dietary...
amino acid levels. The 0.420% of group C, which was the diet of group B plus nonessential amino acids, approximated the 0.486% of group A rather than the 0.270% of group B. The requirement of group C expressed as A/E ratio was 0.119, which was higher than the 0.073 for group A and the 0.080 for group B. This illustrates that the requirement should be elevated in the ratio of methionine to essential amino acid for maximum growth.

Here, it may safely be said that the requirement expressed as dietary percentage, A/E ratio, and A/TN ratio changed according to not only essential amino acids but also to nonessential amino acids. It is considered that body protein gain (N × 6.25) is better than weight gain as a criteria for amino acid requirement. And significant rectilinear relations were found between amino acid intakes and body protein gain for groups by modifying the total nitrogen levels and essential amino acid levels. However, no significant difference was found between the regression lines by the statistical analysis using F-test on variance and t-test on slope and Y-intercept. In other words it may safely be said that these regression lines were considered a regression equation independent of total amino acid level. In general, the amino acid requirements for growing rats have been determined by using maximum growth as the criteria. But maximum growth rate depends on the experimental method, experimental diet, and experimental period. We considered that the requirement for essential amino acid should be increased following body protein gain depending on the different stages of growth for the growing animal increasing the body protein. If the amino acid requirement per one gram of body protein gain is estimated, we can estimate the amino acid requirement depending on the growth rate.

Furthermore, we investigated the relationship between amino acid intake and body protein gain, and determined that 6.2 mg lysine and 9.0 mg of methionine were required for the maintenance of rats weighing 80 g and that 71.6 mg of lysine and 48.8 mg of methionine were required for one gram of body protein gain. Lysine and methionine intakes required for one gram of protein gain were equivalent to 100% and 124% of amino acid contents of body protein (16), respectively. And then the amino acid requirement pattern can be predicted in different the amino acid composition of carcass. It should be emphasized that the requirement with the slopes of the dose-response line is markedly independent of the dietary amino acid level but maximum growth is dependent upon various factors. The requirement varies according to growth rate, application of this estimation of the amino acid requirement per unit of body protein gain will facilitate further study of essential amino acids in many other laboratories.

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