Nitrogen Balance and Body Weight Change of Adult Roosters Fed Single Essential Amino Acid Devoid Diets

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BLOCK and MITCHELL¹⁾ showed a reasonably high correlation between biological value and calculated percentage deficit of the most limiting amino acid with several dietary proteins in rats. From their observation it has been generally accepted that nutritive quality of dietary protein is determined by the most limiting essential amino acid. ASHIDA and YOSHIDA²⁾, however, noted that the effect of the deficiencies of various single essential amino acids on nitrogen balance of adult rats was differed even when the percentage deficiencies of these amino acids for the maintenance requirement were the same. They suggested that there was the nutritional specificity of essential amino acids in the effects of deficiency.

In chickens, there are some reports which suggested the existence of the specificity of essential amino acid to maintain nitrogen balance. LEVEILLE and FISHER³⁾ reported adult roosters were not in negative nitrogen balance even in the complete absence of lysine from the diet. ISHIBASHI⁴⁾ showed that adult roosters fed a diet in which phenylalanine was completely replaced by tyrosine maintained nitrogen equilibrium for 1 year.

In the present study, to demonstrate the existence of comparative nutritional specificity of essential amino acid in chickens the effect of deprivation of single essential amino acid from a well-balanced amino acid diet on nitrogen balance and body weight change in adult roosters was investigated.

Materials and Methods

Single comb, White Leghorn adult roosters weighing about 1460 to 2040 g were used. At the start of the experiments, roosters were individually weighed and were then selected and assigned to 9 groups of 6 birds (experiment 1) or 5 groups of 6 birds (experiment 2) in such a way that the body weight distribution was uniform for each group. They were kept in individual metabolism cages and given appropriate experimental diets for 7 days. On days 3, 5 and 7 body weight was measured. Excreta was collected for the last 3 days of the experimental period and total nitrogen in the excreta was determined by a Kjeldahl method. Nitrogen balance was calculated by subtracting nitrogen in the excreta from nitrogen consumed.

Table 1 shows the composition of a complete diet which contains all essential amino acids at their requirement levels (ISHIBASHI⁴⁾). Diets used in the experiment 1 were 8 single essential amino acids (histidine, lysine, arginine, threonine, isoleucine, tryptophan,

Table 1. Composition (g/kg) of complete diet

Amino acid mixture ¹	57.9
Vitamin mixture ²	2.0
Mineral mixture ³	56.3
Choline chloride	1.5
Corn oil	30.0
Cellulose	30.0
Sucrose	200.0
Corn starch	to 1,000

1 Contained 1.33 g L-Arginine-HCl, 0.33 g L-Histidine-HCl, 2.00 g L-Lysine-HCl, 0.17 g L-Tryptophan, 0.67 g L-Phenylalanine, 0.67 g L-Threonine, 0.67 g L-Leucine, 1.33 g L-Isoleucine, 1.33 g L-Valine, 1.67 g L-Methionine, 6.67 g Glycine and 41.08 g L-Glutamic acid.

2 OKUMURA *et al.*⁵⁾

3 NESHEIM *et al.*⁶⁾

glycine and methionine) devoid diets and an amino acid-free diet. In the experiment 2, phenylalanine-, leucine-, methionine- and amino acid-free diets were used as experimental diets. Glutamic acid was substituted isonitrogenously for deprived amino acids in the deficient diets. The birds consumed these diets at the rate of 30 g/kg body weight/day and this allotment supplied sufficient amounts of nitrogen (197 mg/kg) and energy (105 kcal metabolizable energy/kg) to maintain nitrogen equilibrium in the adult rooster (ISHIBASHI⁴⁾). Water was given *ad libitum*.

Analysis of variance was used to analyze the data statistically and for comparisons of the individual treatment differences, the Duncan's multiple range test (DUNCAN⁷⁾) was applied.

Results and Discussion

Table 2 gives nitrogen balance and body weight change of the roosters fed single essential amino acid deprived diets. Nitrogen balance of roosters were different depending upon the amino acid which deprived from diets. In experiment 1, the roosters receiving the lysine-free diet showed the least nitrogen loss, and feeding the methionine-free diet resulted in the severest nitrogen loss. In experiment 2 the effect of leucine or phenylalanine deficiency on nitrogen balance was significantly less compared with that of methionine deficiency. ISHIBASHI⁸⁾ studied amino acid requirement for maintenance of adult roosters and reported roosters fed histidine- or phenylalanine-free diet could maintain nitrogen equilibrium. Positive nitrogen balance of adult roosters fed phenylalanine-free diet was also reported by ISHIBASHI and KAMETAKA⁹⁾. LEVEILLE and FISHER³⁾ observed that adult roosters receiving lysine- or histidine-devoid diet were in positive nitrogen balance. In the present study roosters fed histidine-, lysine- and phenylalanine-free diets showed negative nitrogen balance, however, they belong to the least negative nitrogen balance group.

Nitrogen balance of the roosters fed the methionine-free diet was the lowest and worse than that of the birds fed an amino acid-free diet. From the results it can be postulated that when roosters were fed an all amino acid deprived diet, methionine would be the most

Table 2. Effect of single essential amino acid deprivation on nitrogen balance and body weight change in adult roosters

Experiment 1		
Deprived amino acid	Nitrogen balance (mg/day)	Body weight change (g/7 days)
Lys	-188 ^a	-36.0 ^a
Trp	-193 ^a	-64.7 ^{a b c}
His	-215 ^a	-27.7 ^a
Gly	-232 ^a	-79.0 ^{a b c}
Ile	-304 ^b	-63.5 ^{a b c}
Thr	-345 ^{b c}	-57.8 ^{a b}
Arg	-367 ^{b c}	-67.3 ^{a b c}
All	-406 ^c	-106.0 ^{b c}
Met	-488 ^d	-114.7 ^c
Pooled SEM	24.7	16.5
Experiment 2		
Deprived amino acid	Nitrogen balance (mg/day)	Body weight change (g/7 days)
Leu	-238 ^a	-58.7 ^a
Phe	-261 ^a	-64.7 ^{a b}
All	-402 ^b	-113.7 ^b
Met	-485 ^b	-101.7 ^{a b}
Pooled SEM	31.7	17.2

Values are means of 6 birds. Means with unlike superscripts in the same column are significantly different ($P < 0.05$).

limiting amino acid. OKUMURA and MURAMATSU¹⁰⁾ found that supplementation of methionine improved nitrogen balance of chicks fed a protein-free diet. Such phenomenon of methionine in rats has been reported. ASHIDA and YOSHIDA²⁾ showed rats fed a protein-free or methionine-free diet were in a similar negative nitrogen balance. YOKOGOSHI and YOSHIDA¹¹⁾ reported the nitrogen-sparing action of supplemented methionine in rats fed a protein-free diet. These reports suggest that methionine is the most limiting amino acid in protein depleted conditions.

Figure 1 shows body weight change of roosters fed the single essential amino acid deprived diets. On day 3 body weight change of the roosters already varied depending upon the deficient amino acid and thereafter the roosters lost their body weight continually and the difference in body weight change between dietary groups became remarkable in both experiments. Similarly to the result of nitrogen balance, body weight loss of roosters fed the histidine- or lysine-free diet was the least and that of birds fed the methionine-free diet was the most. In a series of studies investigating the maintenance requirement of an essential amino acid for adult roosters, LEVEILLE and FISHER^{3,12)} and LEVEILLE *et al.*¹³⁾ reported that birds fed a histidine- or lysine-free diet could maintain their body weight while the birds fed other essential amino acid-free diets showed negative change in body weight. OUSTERHOUT¹⁴⁾ investigated survival time and biochemical changes in chicks fed diets devoid of different essential amino acids and indicated that chicks fed diets lacking histidine or lysine lost weight

Fig. 1. Body weight change of adult roosters fed single essential amino acid deprived diets.

slower and survived longer compared with the other deficient groups. SAID and HEGSTED¹⁵⁾ observed that adult female rats fed a diet free of methionine plus cystine responded in a similar manner to rats fed a protein-free diet, whereas rats fed a lysine- or leucine-free diet lost less tissue than rats fed a protein-free diet did. The results of the present experiment are in line with those of above workers^{3,12,13,14,15)} who showed that histidine- or lysine-deficiency and methionine plus cystine deficiency had the least and the most effect on body weight change among the essential amino acids, respectively.

OUSTERHOUT¹⁴⁾ showed that chicks fed diets lacking isoleucine lost weight rapidly and caused early death to the same extent as the birds fed methionine plus cystine-free diet did. OKUMURA and MORI¹⁶⁾ reported that in chicks fed the diets 50% deficient in single essential amino acid, body weight change and nitrogen retention was reduced most severely by deficiency of isoleucine. Comparing the severity of isoleucine deficiency, the effects were widely different in young and adult chickens. In the present experiment isoleucine deficiency had the medium effect in adult roosters, however in chicks it had the severest one. The difference of isoleucine deficiency response could not be explained, but isoleucine requirement for the growth and the maintenance might give an answer. From these results, it was shown that equal single deficiencies of essential amino acids had different effects on nitrogen balance and body weight change of the adult roosters and all amino acids tested could be roughly divided into three groups with respect to the degree of effect of deficiency; the first group which had the least effect, lysine, histidine and leucine; the second group which had medium effect, tryptophan, glycine, phenylalanine and isoleucine; the third group which had the severest effect, threonine, arginine and methionine.

Summary

Diets completely devoid of single essential amino acid were fed to adult roosters for 7 days to investigate the effect on nitrogen balance and body weight change. Depending upon the kind of amino acid deprived from the diet the effects were different and the existence of the nutritional specificity of essential amino acid was confirmed. Among the essential amino acids tested, lysine, histidine and leucine had the least effect and threonine, arginine and methionine had the severest one. All other amino acids tested, tryptophan, glycine, phenylalanine and isoleucine, had medium effect of deficiency.

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単一必須アミノ酸完全欠乏飼料を給与した成鶏雄の 窒素出納及び体重の変化

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ニワトリに必須アミノ酸を単独で完全に除いた飼料を給与すると、負の窒素出納、体重の減少を示すが、これら指標への影響の程度が欠乏させるアミノ酸の種類によりどのように変化するかを調査した。

供試鶏には白色レグホーン種成鶏雄を用い、各飼料区6反復となるよう分配した後、代謝ケージ内で7日間単飼した。実験は2回に分けて行ない、成鶏雄の各必須アミノ酸要求量を充たす5.8%アミノ酸混合物を含んだ完全飼料から、実験1では、アルギニン、ヒスチジン、リジン、トリプトファン、スレオニン、イソロイシン、メチオニンを、実験2では、ロイシン、フェニルアラニン、メチオニンをそれぞれ単独で完全に排除した飼料を各羽30 g/kg 体重/日の割合で与え、実験後半3日間の窒素出納及び期間中の体重の変化を測定した。

窒素出納、体重の変化への必須アミノ酸欠乏の影響は

欠乏させるアミノ酸の種類によって様々であり、リジン、ヒスチジンは必須アミノ酸の中でもその欠乏の影響は最も弱く、これに対しメチオニンの欠乏は最も強く、全くアミノ酸を含まない飼料を給与した群と比較しても、ほぼ等しい体重減少とより低い窒素出納を示した。同程度の欠乏であっても欠乏させるアミノ酸の種類によりその影響が異なるというアミノ酸の栄養特異性の存在が確認され、また必須アミノ酸を完全欠乏の影響の程度により3つのグループに大別すると、ヒスチジン、リジン、ロイシンは弱いグループに、トリプトファン、フェニルアラニン、グリシン、イソロイシンは中程度のグループに、スレオニン、アルギニン、メチオニンは強いグループに分けられた。

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