Effect of Excess of Individual Essential Amino Acids in Diets on Chicks

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It has been reported that amino acid toxicity in animals varies with individual amino acids¹⁻³⁾. Toxic effects have been reported by SMITH⁴⁾ for all the amino acids essential for chicks. However, the aim of his report was to determine the alleviating effect of arginine on the toxicities of various amino acids when these amino acids were supplemented at varying levels, and not to investigate and compare the toxicities of these amino acids supplemented at a constant level.

The present experiment was therefore undertaken to investigate the effect of excessive intakes of individual essential L-amino acids and compare their individual characteristics. Hence, we studied the chicks given a diet containing 3% of each of the amino acids, L-valine, L-leucine, L-isoleucine, L-lysine, L-histidine, L-threonine, L-methionine, L-cystine, L-tyrosine, L-phenylalanine, L-tryptophan and glycine.

Materials and Methods

Eight-day-old single comb White Leghorn male chicks were used. After 2 hours fasting they were housed individually in metabolism cages and kept at $28 \pm 2^\circ\text{C}$ in an animal room with continuous lighting. The composition of the basal diet is given in Table 1. Basal diet contained 10% crude protein which was supplied by soybean protein fortified with methionine. Three percent of each amino acid was added to the basal diet at the expense of cornstarch. Diet and water were given *ad libitum*. The experiment was done using

Table 1. Composition of basal diet

Soybean protein (CP 81%)	12.33%
Corn starch	43.44
Sucrose	20.00
Corn oil	3.00
Mineral mixture ¹⁾	5.63
Cellulose	5.00
L-Methionine	0.25
Vitamin mixture ²⁾	0.20
Choline chloride	0.15
Aluminium silicate	10.00

1) NESHEIM *et al.* (6)

2) OKUMURA *et al.* (7)

4 chicks to each dietary treatment for 10 days; body weight and food intake were determined on alternate days.

At the end of the 10 d. experimental period, blood samples were taken by cardiac puncture using a heparinized syringe, transferred to a centrifuge tube and immediately centrifuged to separate the plasma. Four samples of each treatment were pooled and deproteinized with 1% picric acid. The amino acid analysis of blood plasma was performed by the method of MOORE *et al.*⁵⁾ using automatic ion-exchange chromatography (JEOL Model JLC-5AH, Japan Electron Optics Laboratory Co. Ltd., Tokyo) for the treatment of tyrosine, phenylalanine, valine, leucine, isoleucine and control.

Results and Discussion

Table 2 gives the effect of feeding excessive single amino acids in a low protein diet on growth. Diets containing excess L-arginine, glycine, L-valine, L-cystine and L-isoleucine showed little or slight growth inhibition. Diets containing excess L-leucine, L-tyrosine, L-histidine, L-lysine, L-threonine and L-tryptophan showed apparent growth inhibition. The severest growth inhibition was caused by L-phenylalanine- and L-methionine-excess diets. L-Methionine was the most toxic of all amino acids tested: only in this group was body weight reduced and two out of four chicks died during the course of the experiment. Diet containing excess L-arginine caused no growth depression and had the tendency of improving body weight gain and food efficiency. Though the food intakes was depressed in the chicks given the diet containing excess glycine, growth of this group was not depressed and food efficiency was improved significantly ($P < 0.05$). The improvement of

Table 2. Effect of 3% single amino acid supplementation on chicks fed a 10% protein diet

Diet	Body weight gain g/10 days	Food intake g/10 days	Food efficiency %	Growth depression ¹⁾ %
Basal	58 ± 1 ^a	179 ± 3	32 ± 1	0
L-Arginine	65 ± 5	165 ± 6	39 ± 2	-12
Glycine	57 ± 3	139 ± 8 ^b	41 ± 1 ^a	2
L-Valine	55 ± 4	166 ± 5	33 ± 2	5
L-Cystine	50 ± 3	142 ± 6 ^a	35 ± 1	14
L-Isoleucine	48 ± 3	148 ± 7	32 ± 1	17
L-Leucine	43 ± 7	136 ± 10 ^b	31 ± 3	26
L-Tyrosine	41 ± 4	151 ± 12	27 ± 1	29
L-Histidine	40 ± 4 ^a	132 ± 7 ^b	30 ± 2	31
L-Lysine	35 ± 2 ^b	129 ± 6 ^b	27 ± 1	40
L-Threonine	29 ± 2 ^b	106 ± 4 ^b	27 ± 1	50
L-Tryptophan	24 ± 2 ^b	106 ± 4 ^b	22 ± 1 ^b	59
L-Phenylalanine	7 ± 1 ^b	80 ± 3 ^b	8 ± 1 ^b	88
L-Methionine*	-14 ± 3 ^b	40 ± 2 ^b	-34 ± 5 ^b	124

1) Percentage growth depression = $100 - (\text{body weight gain of each amino acid-supplemented group} / \text{body weight gain of control}) \times 100$.

2) Mean ± SE. Statistical significance: a, different from control at $P < 0.05$; b, different from control at $P < 0.01$.

* Two missing values.

growth and food efficiency in the arginine group, or improvement of food efficiency in the glycine group indicate that added arginine or glycine could be used as a nitrogen sources for chicks given a low protein diet. Arginine and glycine may be serving as sources of non-specific N by transamination and improving the amino acid balance with respect to essential: non-essential amino acid ratio. It rather depends on the mechanism of toxicity. Glycine may be assisting in excretion of excess ammonia (toxic) produced from excess amino acids. VISEK⁷⁾ showed arginine could prevent toxic effect of ammonia in rats because it stimulated synthesis of N-acetylglutamate needed for urea synthesis, but this seems likely to be less important in chicks which only have low urea cycle activity.

Significant growth depressions were observed in chicks given L-lysine, L-threonine, L-tryptophan, L-phenylalanine or L-methionine excess diets ($P < 0.01$). Significant food intake depressions were observed in chicks given L-leucine, L-tyrosine, L-histidine, L-lysine, L-threonine, L-tryptophan, L-phenylalanine or L-methionine excess diets ($P < 0.01$). Food efficiency was depressed significantly in chicks given L-tryptophan, L-phenylalanine or L-methionine excess diets ($P < 0.01$).

Considering the growth depression and food intake in the present experiment, all amino acids tested could be roughly divided into three groups with respect to the degree of toxic effect on chicks: the first group which causes no or slight depression, L-arginine, glycine, L-valine, L-cystine and L-isoleucine; the second group which causes a marked depression, L-leucine, L-tyrosine, L-histidine, L-lysine and L-threonine; the third group which causes the severest depression, L-tryptophan, L-phenylalanine and L-methionine.

It is of interest to note that though the structure of cystine resembles that of methionine or that of tyrosine resembles phenylalanine, cystine and tyrosine belong to less toxic group and methionine and phenylalanine to the most toxic group.

MURAMATSU *et al.*³⁾ reported that when the rats were given a 10% casein diet containing 5% individual amino acids all amino acids tested could be divided into three groups. The first group, producing no or slight growth depression, was leucine and arginine. The second group, which caused a marked growth depression, was isoleucine, lysine, valine, threonine, glycine, cystine, tryptophan and histidine. The third group, which caused more severe growth depression, was tyrosine, phenylalanine and methionine. Though the present experiment roughly agrees with the result of MURAMATSU *et al.*³⁾, some amino acids (notably glycine and cystine which caused no or slight depression and tyrosine which caused not so severe depression in chicks) are different. This suggests that though the supplemental level of amino acids, the quality of diet, etc. were different, amino acid toxicity in chicks may be somewhat different from that in rats.

The concentration of free amino acids in the plasma of chicks given the diets supplemented high levels of a single amino acid are given in Table 3. Though an increase in the plasma free amino acid which was added to the diet was common to the all amino acids tested, the extent of that amino acid varied with individual amino acids. The plasma free amino acid concentration of the amino acid supplemented was more than twenty times as much as that of control in the L-valine, L-tyrosine and L-phenylalanine groups, but only about twice as much as that of control in the leucine group. A large increase in tyrosine and not only phenylalanine in plasma was observed in the phenylalanine suppl-

Table 3. Concentrations of plasma amino acids of chicks fed 3% single amino acid excess diets (μ moles per 100 ml)¹⁾

Diet	Lys	His	Arg	Thr	Ser	Pro	Gly	Val	Met	Ile	Leu	Tyr	Phe
Basal	32	12	19	25	57	20	32	12	13	18	16	16	10
Val	74	14	13	42	97	30	32	292	15	21	18	23	11
Leu	48	11	18	55	81	27	35	7	14	11	36	14	9
Ile	45	9	11	44	84	32	32	8	11	72	13	30	8
Tyr	78	9	12	49	122	37	33	15	16	21	25	355	—
Phe	62	13	18	9	101	30	52	18	23	19	14	110	227

1) Pooled samples from four chicks.

emented group, but as the peak of phenylalanine was masked by the greatly increased adjacent tyrosine peak in the tyrosine group, the precise concentration of plasma free phenylalanine could not be determined in this group. It is supposed that as tyrosine is produced by the metabolism of phenylalanine a large increase in plasma free tyrosine in the phenylalanine group may be attributed to the tyrosine synthesized from phenylalanine. Decrease in valine and isoleucine in plasma were observed in the leucine group, and decrease in valine in the isoleucine group, but such an effect was not observed in the valine group. With regard to valine, leucine and isoleucine, a similar effect was reported in rats given a diet containing excess leucine^{9,10)}. It is therefore possible that these effect may indicate the presence of a branched chain amino acid antagonism in chicks and this may require further investigation.

Summary

Excesses of single amino acids given in the diets of chicks from day 8 to day 18 depressed growth and food intake to varying degrees, which were dependent on the kind of amino acid supplemented. L-Methionine and L-phenylalanine caused the severest growth depression. L-Tryptophan, L-threonine, L-lysine, L-histidine, L-tyrosine and L-leucine were apparently toxic. All other amino acids tested, L-arginine, glycine, L-valine, L-cystine and L-isoleucine, showed no or only a slight effect.

An increase in the blood plasma concentration of the amino acid in dietary excess was observed, and the possible existence of a branched-chain amino acid antagonism was suggested in chicks.

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飼料中のアミノ酸の単一過剰がニワトリヒナに及ぼす影響

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ニワトリにメチオニンなどのアミノ酸を単一に過剰に給与すると過剰障害のあることが知られているが、アミノ酸の比較についてはあまり知られていない。そこで本実験ではニワトリヒナを用いて各種アミノ酸の単一過剰障害を増体量などを指標として比較した。

供試ヒナは、体重の均一な8日齢の白色レグホーン種雄を用い、各飼料区4反復として代謝ケージで10日間単飼した。蛋白質源としてメチオニンを補足した大豆蛋白質を用い、粗蛋白質10%の飼料を対照飼料とし、これに単一アミノ酸を添加して、各種アミノ酸の単一過剰障害を比較調査した。過剰に用いたアミノ酸は、グリシン、バリン、ロイシン、イソロイシン、アルギニン、リジン、ヒスチジン、スレオニン、メチオニン、シスチン、チロシン、フェニルアラニン、トリプトファン¹⁾の13種である。

過剰のアミノ酸によるヒナの成長の低下とそれによる

過剰障害の程度は、アミノ酸の種類によって様々であった。メチオニンは最も過剰障害が強く、増体量が負となった。一方、アルギニンとグリシンは全く障害がないばかりか、対照飼料が10%蛋白質飼料ということもあり、増体量あるいは飼料効率を改善した。今回調査したアミノ酸は増体量、飼料摂取量および飼料効率からみると、アミノ酸の単一過剰障害は大別して3つのグループに分けられ、アルギニン、グリシン、バリン、シスチン、イソロイシンは過剰障害の弱いグループ、ロイシン、チロシン、ヒスチジン、リジン、スレオニンは中程度のグループ、トリプトファン、フェニルアラニン、メチオニンは強いグループに属した。

血漿中の遊離アミノ酸の濃度は、飼料中に添加されたアミノ酸が上昇することが認められ、ロイシンの過剰によって血漿中のイソロイシンとバリンが減少する傾向が認められた。(家禽会誌, **17**, 135~139, 1980)