Influence of Dietary Supplementation of Arginine or Glycine on the Adverse Effect in Chicks Given a Tyrosine Excess Diet

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When chicks are given a diet containing an excessive amount of tyrosine, their growth is retarded¹⁻⁴⁾ and they develop lesions of the feet and eyes⁴⁾. These adverse effects can be overcome by an addition of dietary protein⁴⁾. Some attempts have been made to alleviate growth-depressing effect of excess tyrosine in chicks by supplemental arginine⁵⁻⁷⁾. SMITH⁶⁾ showed that growth depression in chicks given a basal diet containing 12% crude protein from casein supplemented with 3% tyrosine was significantly alleviated by the addition of 0.6 to 1.5% arginine · HCl. SNETSINGER and SCOTT⁸⁾ reported the ameliorative effect of supplemental arginine and/or glycine on growth depression due to dietary excess of single supplemental amino acid, *i. e.*, lysine, histidine and phenylalanine. However, more detailed studies on the effect of arginine or glycine have not as yet been done.

As part of a study aimed at determining the factors that can alleviate the detrimental effect of excess tyrosine, we examined the effects of supplemental arginine or glycine on growth, nitrogen utilization, free amino acid concentration in plasma and excretion of some nitrogenous compounds in droppings of chicks given a 5% tyrosine diet.

Experimental procedure

Day-old Single Comb White Leghorn male chicks were housed in electrically-heated brooders. They received a commercial chick mash for 7 days. On day 8 the chicks were individually weighed after having been fasted for 2 hours and were then selected and distributed, based on their body weights, in 12 groups of five chicks each. They were then housed individually in metabolism cages⁹⁾ for the measurement of feed consumption and quantitative collection of droppings. Eleven groups of five chicks each were fed their corresponding experimental diets *ad libitum* from day 8 to day 22. Water was made available at all times. The remaining five chicks of the last group were killed with diethyl ether and used to determine the initial body composition.

The basal diet (20% crude protein content) contained, in percentage by weight, isolated soybean protein, 24.93; sucrose, 20.00; aluminum silicate, 10.00; cellulose, 5.00; corn oil, 3.00; L-methionine, 0.10; choline chloride, 0.15; vitamin mixture⁹⁾, 0.20; mineral mixture¹⁰⁾, 5.63 and cornstarch, make up to 100. The chick's requirement for methionine¹¹⁾ was just met by the supplementary level indicated. Tyrosine excess diet was prepared by adding 5.0% L-tyrosine to the basal diet at the expense of cornstarch. Arginine or glycine supplemented diets were made by adding 0.5, 1.0, 1.5, 2.0 and 2.5%

of L-arginine · HCl and 1.0, 2.0, 3.0 and 4.0% of glycine, respectively, to the tyrosine excess diet at the expense of cornstarch. When the experimental diet containing L-arginine · HCl was prepared, appropriate amounts of sodium bicarbonate were added to neutralize the hydrochloride in arginine.

The chicks were weighed and feed consumption was measured every other day. The droppings from each chick were collected into 100 ml 0.1 N hydrochloric acid in deep, stainless steel trays beneath the metabolism cages. The acid prevented further microbial action and loss of ammonia in the droppings. Droppings collected daily during the last 4 days were air-dried at 60°C and used for the determinations of total nitrogen, uric acid and total creatinine. Total nitrogen was analyzed by the Kjeldahl method and uric acid was determined according to PUDELKIEWICZ *et al.*¹²⁾ and total creatinine was determined spectrophotometrically by the Jaffé reaction¹³⁾.

At the end of the 14-day experimental period, blood was obtained in heparinized syringe by cardiac puncture. Plasma was separated by centrifugation and deproteinized with 5 volumes of 1% picric acid solution. The deproteinized plasma samples were frozen and kept in that state until they were analyzed for free amino acids. Before analysis equal volumes of deproteinized plasma samples from five chicks within each treatment were pooled. Amino acid was determined with the amino acid autoanalyzer, JEOL model JLC-5AH. All chicks were killed for carcass analysis, at the end of the experiment. Carcasses were treated according to the procedure described by OKUMURA and MORI¹⁴⁾. Nitrogen retention during the experimental period was estimated by subtracting the initial values (day 8) of body composition from the final ones (day 22). Results were subjected to an analysis of variance. Comparison of treatment means was tested by DUNCAN's multiple range method¹⁵⁾. More generally, the variability of each treatment mean is indicated by the standard error of mean.

Results

Table 1 gives the effect of dietary supplementation of arginine or glycine on body

Table 1. Effect of dietary supplementation of arginine or glycine on body weight gain, feed intake and gain:feed ratio in chicks given a 5% tyrosine diet (Mean±SEM for 5 birds)

Diet	Body weight gain	Feed intake	Gain:feed
	g/bird/14 days		
Basal (B)	118.6±5.9 ^a	262.6± 8.3 ^a	0.451±0.015 ^a
B+5.0% L-Tyr (T)	60.4±5.2 ^b	184.2± 8.6 ^b	0.326±0.014 ^b
T+0.5% L-Arg·HCl	61.2±6.5 ^b	186.8± 7.6 ^b	0.327±0.028 ^b
T+1.0% L-Arg·HCl	62.2±5.8 ^{bc}	193.4± 8.7 ^b	0.320±0.017 ^b
T+1.5% L-Arg·HCl	78.6±6.8 ^{bc}	228.8±10.3 ^c	0.342±0.016 ^b
T+2.0% L-Arg·HCl	81.6±5.8 ^{cd}	228.0±12.0 ^c	0.356±0.007 ^{bc}
T+2.5% L-Arg·HCl	74.6±6.2 ^{bc}	211.6± 5.3 ^{bc}	0.351±0.022 ^{bc}
T+1.0% Gly	62.0±5.6 ^{bc}	193.0±11.1 ^b	0.319±0.011 ^b
T+2.0% Gly	68.8±4.8 ^{bc}	196.0± 8.6 ^b	0.350±0.012 ^{bc}
T+3.0% Gly	71.4±7.3 ^{bc}	198.6±10.4 ^b	0.357±0.023 ^{bc}
T+4.0% Gly	85.6±6.5 ^d	213.4± 8.6 ^{bc}	0.399±0.015 ^c

Values without a common letter indicate a significant difference ($P < 0.05$).

weight gain, feed intake and gain:feed ratio of chicks given a 5% tyrosine diet. The addition of 5% tyrosine to the basal diet resulted in a significant ($P<0.05$) depression in body weight gain, feed intake and gain:feed ratio. The depressed body weight gain, feed intake and gain:feed ratio had a tendency to increase in proportion to the amount of arginine•HCl (up to 2.0%) and glycine supplemented. Such improvements were statistically significant ($P<0.05$) when arginine•HCl was 2.0% and glycine 4.0%. Similarly, the addition of 1.5 and 2.0% arginine•HCl significantly ($P<0.05$) alleviated the depressed feed intake, and the addition of 4.0% glycine significantly ($P<0.05$) improved the gain:feed ratio. Significant ($P<0.01$) correlation coefficients ($r=0.8898$ for arginine supplemented groups and $r=0.9187$ for glycine supplemented groups) were obtained between body weight gain and feed intake.

Table 2. Effect of dietary supplementation of arginine or glycine on nitrogen utilization in chicks given a 5% tyrosine diet (Mean $\pm$ SEM for 5 birds)

Diet	N intake	N retention	N retention rate
	g/bird/14 days		
Basal (B)	8.67±0.27 ^a _{de}	3.63±0.16 ^a	0.419±0.015 ^a
B + 5.0 % L-Tyr (T)	6.63±0.31 ^b	2.23±0.15 ^{bc}	0.336±0.013 ^b
T + 0.5 % L-Arg·HCl	6.91±0.28 ^{bc}	2.19±0.13 ^{bc}	0.317±0.015 ^{bc}
T + 1.0 % L-Arg·HCl	7.16±0.32 ^{bc}	2.21±0.17 ^{bc}	0.307±0.010 ^{bc} _d
T + 1.5 % L-Arg·HCl	8.47±0.38 ^a _{de}	2.52±0.11 ^c _d	0.297±0.005 ^c _d
T + 2.0 % L-Arg·HCl	9.12±0.48 ^a	2.56±0.06 ^c _d	0.283±0.011 ^c _d
T + 2.5 % L-Arg·HCl	8.46±0.21 ^a _{de}	2.30±0.15 ^{bc}	0.271±0.012 ^d
T + 1.0 % Gly	7.14±0.41 ^{bc}	2.04±0.16 ^b	0.285±0.007 ^c _d
T + 2.0 % Gly	7.64±0.34 ^{bc} _d	2.20±0.10 ^{bc}	0.288±0.005 ^c _d
T + 3.0 % Gly	7.94±0.42 ^c _{de}	2.27±0.20 ^{bc}	0.286±0.021 ^c _d
T + 4.0 % Gly	8.96±0.36 ^a _e	2.76±0.15 ^d	0.308±0.006 ^{bc} _d

Values without a common letter indicate a significant difference ($P<0.05$).

Table 3. Effect of dietary supplementation of arginine or glycine on the excretion of some nitrogenous compounds in excreta of chicks given a 5% tyrosine diet (Mean $\pm$ SEM for 5 birds, mg/bird/4 days)

Diet	N intake	N excreted	Uric acid N		Total creatinine N	
Basal (B)	2884 $\pm$ 170	1693 $\pm$ 89	814 $\pm$ 47*	(48.3)**	12.0 $\pm$ 0.6**	(0.7)**
B+5.0% L-Tyr(T)	2419 $\pm$ 283	1606 $\pm$ 117	640 $\pm$ 37	(41.0)	27.7 $\pm$ 2.1	(1.7)
T+0.5% L-Arg•HCl	2227 $\pm$ 100	1608 $\pm$ 50	705 $\pm$ 23	(44.1)	27.1 $\pm$ 2.3	(1.7)
T+1.0% L-Arg•HCl	2205 $\pm$ 184	1786 $\pm$ 132	806 $\pm$ 29*	(46.1)	26.8 $\pm$ 2.0	(1.5)
T+1.5% L-Arg•HCl	2694 $\pm$ 165	1977 $\pm$ 150	900 $\pm$ 62**	(45.6)	24.2 $\pm$ 1.5	(1.2)**
T+2.0% L-Arg•HCl	3080 $\pm$ 272	2127 $\pm$ 115	993 $\pm$ 54**	(47.0)*	23.8 $\pm$ 1.3	(1.1)**
T+2.5% L-Arg•HCl	2824 $\pm$ 117	2124 $\pm$ 115	973 $\pm$ 39**	(44.8)	24.0 $\pm$ 2.9	(1.1)**
T+1.0% Gly	2311 $\pm$ 149	1690 $\pm$ 65	823 $\pm$ 23**	(50.8)**	27.0 $\pm$ 1.3	(1.6)
T+2.0% Gly	2551 $\pm$ 110	1759 $\pm$ 37	833 $\pm$ 34**	(47.6)*	30.3 $\pm$ 1.8	(1.6)
T+3.0% Gly	2760 $\pm$ 158	2016 $\pm$ 89	1035 $\pm$ 48**	(51.4)**	33.9 $\pm$ 3.7	(1.7)
T+4.0% Gly	3032 $\pm$ 216	2068 $\pm$ 95	1202 $\pm$ 68**	(58.1)**	34.0 $\pm$ 1.8	(1.7)

Values with asterisk are significantly different from T group: ** $P<0.01$, * $P<0.05$. Numbers in parentheses represent means expressed as a percentage of N excreted.

Table 2 gives the effect of dietary supplementation of arginine or glycine on nitrogen utilization in chicks given a 5% tyrosine diet. The addition of excess tyrosine resulted in a significant ($P < 0.05$) depression in nitrogen retention rate. The addition of 2.0% arginine·HCl or 4.0% glycine to the tyrosine excess diet caused a significant ($P < 0.05$) increase in nitrogen intake and the supplementation of 4.0% glycine resulted in a significant ($P < 0.05$) increase in nitrogen retention. A significant ($P < 0.05$) decrease in nitrogen retention rate was obtained when 1.5, 2.0 and 2.5% of arginine·HCl or 1.0, 2.0 and 3.0% of glycine were supplemented.

Table 3 shows the effect of dietary supplementation of arginine or glycine on the excretion of some nitrogenous compounds in excreta of chicks given a 5% tyrosine diet. Feeding the excess tyrosine diet resulted in a significant ($P < 0.05$) decrease in the excretion of uric acid nitrogen, while the excretion of total creatinine, to a lesser extent, increased significantly ($P < 0.01$). The addition of arginine or glycine counteracted the depression of the proportion of uric acid in nitrogen of excreta that had been caused by giving excessive amounts of tyrosine.

Table 4 shows the effect of dietary supplementation of arginine or glycine on free amino acid concentrations in plasma of chicks given a 5% tyrosine diet. The plasma concentrations of arginine and threonine were reduced in chicks given excess tyrosine. In contrast, there was a highly significant increase in tyrosine concentration, which was elevated about 30-fold. When 2.0% arginine·HCl or 4.0% glycine was included into the tyrosine excess diet, the plasma tyrosine concentration was moderately reduced. The concentration of plasma phenylalanine also underwent a change similar to that of the plasma tyrosine.

The appearance of external foot lesions⁴⁾ was observed in chicks given a tyrosine excess diet in two out of five chicks. In chicks that received a tyrosine excess diet containing 1.5, 2.0% arginine·HCl or 4.0% glycine, external foot lesions were not observed. There was not, however, a close relationship between the supplementation of arginine or glycine and the occurrence of signs of tyrosine toxicity.

Table 4. Effect of dietary supplementation of arginine or glycine on free amino acid concentrations in plasma of chicks given a 5% tyrosine diets¹

Diet	Amino acid	Lys	Arg	Thr	Gly	Val	Met	Ile	Leu	Tyr	Phe
		$\mu\text{mol}/100\text{ ml plasma}$									
Basal (B)		105	34	120	75	32	9	22	25	21	18
B + 5.0% L-Tyr (T)		95	16	74	66	40	6	24	24	634	61
T + 0.5% L-Arg·HCl		132	18	54	52	19	8	16	18	519	47
T + 1.0% L-Arg·HCl		99	35	51	68	38	10	27	26	633	82
T + 1.5% L-Arg·HCl		125	43	66	72	40	10	26	27	629	82
T + 2.0% L-Arg·HCl		123	41	51	64	36	5	19	24	442	38
T + 2.5% L-Arg·HCl		152	64	79	87	48	8	29	32	673	63
T + 1.0% Gly		110	32	66	155	47	9	33	34	696	89
T + 2.0% Gly		94	34	73	189	38	11	27	27	627	54
T + 3.0% Gly		109	38	77	250	45	13	34	28	589	67
T + 4.0% Gly		77	23	70	286	29	10	22	22	375	46

1. Samples were pooled from 5 chicks/group.

Discussion

The beneficial effect of the limiting amino acid for the growth of young rats against the tyrosine toxicity has been reported¹⁶⁾. In the present experiment, nitrogen retention rate was not improved by the supplementation of arginine or glycine and which suggested that the ameliorative effect of arginine or glycine was done in some way(s) unrelated to its role as the limiting amino acid. The evidence indicated that the improvement of the depressed body weight gain in the arginine supplemented groups primarily depends on the increment in feed intake, while in the glycine supplemented groups, on the improvement of gain: feed ratio. A difference of the effect on chick's performance between arginine and glycine supplementation may reflect the different metabolic function of both amino acids. It is conceivable that the recovery of the depressed feed intake, which resulted in the improvement of growth depression, augmented the resistance of chicks to excess tyrosine, because a close correlation between body weight gain and feed intake was observed.

It has been shown that feeding chicks a diet containing an excessive amount of tyrosine increased the chick's requirement for arginine^{6,7)}. AUSTIC and NESHEIM⁷⁾ showed that a tyrosine excess diet augmented arginine requirement of chicks by enhancing the kidney arginase activity. Glycine is one of the substrates for uric acid biosynthesis¹⁷⁾ and supplemental glycine might function in alleviation of the tyrosine toxicity by enhancing the excretion of nitrogen through the uric acid formation. The observation that the addition of glycine largely increased the proportion of uric acid in total nitrogen of excreta might support this hypothesis. The other reason why supplemental arginine or glycine was effective to alleviate the adverse effect of chicks given a tyrosine excess diet might relate to the increased creatinine excretion. The domestic fowl excretes aromatic compounds as ornithuric acid, which is conjugated aromatic compounds with ornithine¹⁸⁾. The required ornithine could be supplied through the reaction forming urea and the transamidation reaction forming creatine¹⁷⁾. One could, therefore, postulate that unabsorbed tyrosine was degraded by the intestinal bacteria and then excreted as ornithuric acid⁵⁾. BAKKE¹⁹⁾ identified various simple phenols in the urine of rats given a 10% tyrosine diet and suggested that production of these compounds may contribute to the manifestation of tyrosine toxicity.

In the present experiment, plasma tyrosine concentration of chicks given a 5% tyrosine diet supplemented with 4.0% glycine decreased to about 60% of plasma tyrosine of chicks given a 5% tyrosine diet alone. ALAM *et al.*²⁰⁾ reported that dietary addition of glycine to a diet containing excess tyrosine caused an increase in tyrosine aminotransferase activity and which accompanied by a substantial decrease in plasma tyrosine concentration. Further investigation will be required to clarify the relationship between dietary glycine and tyrosine aminotransferase activity in chicks under circumstance of excess tyrosine.

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Summary

Experiment was done to determine whether arginine or glycine was required to improve

the adverse effect of a tyrosine excess diet. Chicks given the tyrosine excess diet demonstrated the retarded growth, depressed feed intake and decreased gain:feed ratio. These indices had a tendency to increase with the level of arginine · HCl (up to 2.0%) or glycine supplementation. The addition of 1.5 and 2.0% arginine · HCl to the tyrosine excess diet significantly ($P < 0.05$) increased the feed intake and the addition of 4.0% glycine significantly ($P < 0.05$) improved the gain:feed ratio. The decreased nitrogen retention rate, which was caused by feeding the tyrosine excess diet, was not improved by the supplementation of arginine or glycine. Chicks given the tyrosine excess diet significantly ($P < 0.01$) increased the excretion of total creatinine. Supplemental arginine or glycine did not result in a substantial change in the increased excretion of total creatinine. The free tyrosine concentration in plasma was remarkably elevated by feeding excess tyrosine. When 2.0% arginine · HCl or 4.0% glycine was added to the tyrosine excess diet, plasma tyrosine concentration was slightly lower than that of chicks given the tyrosine excess diet. The results showed that the beneficial effect of supplemental arginine or glycine on the growth depression due to excess tyrosine could be attributed to the significant improvement of feed intake or gain:feed ratio.

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ニワトリヒナにおけるチロシン過剰障害におよぼす アルギニンあるいはグリシン添加の影響

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単一アミノ酸を過剰に含む飼料をニワトリヒナに給与すると、成長の抑制や飼料摂取量の減少がみられ、これらの害作用はアルギニンやグリシンの添加によって緩和されることが知られている。本実験では、チロシン過剰飼料給与によって生じた成長抑制におよぼすアルギニンあるいはグリシンの緩和効果について、増体量、飼料摂取量、飼料効率、血漿遊離アミノ酸濃度および窒素の利用性などを指標として研究した。

試験飼料は、蛋白質源としてL-メチオニンを補足した大豆蛋白質を用いて基礎飼料（20% CP）を調製し、これに5%のL-チロシンを添加してチロシン過剰飼料とした。さらにチロシン過剰飼料に0.5, 1.0, 1.5, 2.0および2.5%のL-アルギニン塩酸塩（以下アルギニンと略す）を、また、1.0, 2.0, 3.0および4.0%のグリシンをそれぞれ添加してアルギニン添加飼料およびグリシン添加飼料を調製した。供試ヒナは8日齢の白色レグホーン種雄を用い、14日間試験飼料および水を自由摂取させた。

チロシン過剰飼料をヒナに給与すると基礎飼料に比べ、増体量、飼料摂取量および飼料効率が著しく減少した。これらの諸項目は、添加したアルギニン（2.0%まで）あるいはグリシンの増加に比例して増加する傾向がみられた。増体量は2.0%のアルギニンおよび4.0%の

グリシンの添加によって、飼料摂取量は1.5および2.0%のアルギニンの添加によって、飼料効率は4.0%のグリシンの添加によって有意な改善がみられた（ $P < 0.05$ ）。窒素蓄積率（窒素蓄積量/窒素摂取量）は、チロシン過剰飼料給与により著しい減少がみられ、これはアルギニンあるいはグリシンの添加によって改善されなかった。チロシン過剰飼料の摂取は、尿酸態窒素排泄量の減少をもたらしたが、一方総クレアチニン態窒素の排泄量を増加させた。アルギニンあるいはグリシンの添加により尿酸態窒素排泄量は基礎飼料の排泄量より増加したが、総クレアチニン態窒素排泄量に変化はみられなかった。血漿遊離チロシン濃度は、チロシン過剰飼料給与によって著しく増加したが、アルギニンあるいはグリシンの添加によって幾分減少の傾向がみられた。また血漿遊離フェニルアラニン濃度は、遊離チロシン濃度の変化と同様の傾向を示した。

これらの結果から、チロシン過剰飼料給与によって生じた成長抑制に対するアルギニンあるいはグリシンの緩和効果は窒素蓄積率の改善によるのではなく、飼料摂取量の増加あるいは飼料効率の改善によることが示された。

（家禽会誌, 18, 151～157, 1981）