Influence of Excessive Single Amino Acid Intake to Free Amino Acid Potencies of Chick Organs

I. Excessive Glycine and Free Amino Acid Patterns of Chick Liver

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Since ALMQUIST *et al.*^{1,2)} reported that glycine was an amino acid required by chick for growth, importance of this amino acid in poultry feeding has been emphasized and the biochemical concerns of glycine and its related compounds were also studied by WIXOM *et al.*³⁾. On the other hand, the free amino acid level of animal body fluids and organ tissues were appointed as an useful index in the field of protein nutrition, as LONGENECKER *et al.*⁴⁾ proposed a technique to estimate the adequacy of amino acids of dietary protein through measuring the potency of the amino acids in question in plasma of the test animals. The present study was so conducted to inform the free amino acid variety under influence of glycine supplementation to a chick diet.

Experimentals

A commercial chick feed (table 1) was given to Plymouth Rock male×White Leghorn female crossbred cockerels. Sixty five test chicks of similar body weight were selected for the experiment comprising five groups differing in feed composition (tables 1 and 2). Livers of the test chick (table 3) were subjected to free amino acid assay. Each group including 10 chicks (commercial feed group, 25 chicks) was housed in a metal cage, room temperature

Table 1. Amino acid contents of the commercial chick feed (g. of each constituent in 100 g. feed)

Isoleucine	0.88	Histidine	0.99
Leucine	1.6	Glutamic acid	3.2
Lysine	1.0	Glycine	1.1
Methionine	0.29		
Phenylalanine	1.1	Crude protein	21.0
Threonine	0.71	Crude fat	3.0
Tryptophan	0.31	Crude fibre	7.2
Arginine	1.1		

Six N hydrochloric acid was used to obtain hydrolysate of powder of the feed with an exception that 4 N sodium hydroxide containing L-cystein hydrochloride was applied to hydrolyse for tryptophan assay.

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Table 2. The composition of the basal feed (g. of each constituent in 100 g. feed)

Constituents	Period of supply	First to 3rd exptl. weeks		Fourth to 5th exptl. weeks	
	Level of protein	16%	20%	16%	20%
Maize		64.0	58.0	66.0	58.0
Wheat		9.5	9.5	12.0	12.0
Wheat bran		5.0	5.0	5.0	5.0
Soybean meal		15.0	15.0	11.0	11.0
Fish meal		2.0	8.0	1.5	9.5
Alfalfa meal		3.0	3.0	3.0	3.0
Supplemented vitamins, minerals <i>etc.</i> *		1.5	1.5	1.5	1.5
Crude protein		16.1	21.1	15.8	21.9
Glycine(assayed)		0.97	1.1	1.0	1.1

For preparation of glycine added feeds, 4g of reagent grade glycine and 96g of the above specified basal feeds were mixed.

* The following substances were contained in 1.5g of the supplementary compartment: 0.80g of calcium-carbonate, 0.25g of sodium chloride, 0.30 mg of thiamine, 1.0 mg of riboflavin, 0.20 mg of nicotinic acid, 14 mg of choline chloride, 0.55 mg of calcium pantothenate, 0.10 mg of folic acid, 25,000 IU of vitamin A, 5,000 IU of vitamin D₃, 2.2 mg of chlortetracyclin (drug), 0.10 g of nitrofurantoin deriv. (*anti* coccidiosis drug), 31 mg of Cu, 300 mg of Fe, 20 mg of Zn, 3 mg of Co and 830 mg of Mn.

Table 3. The test chick groups for the liver amino acid assay (liver of the chicks fed the diet tabulated here)

(I) Commercial chick feed* group (one week after hatching)	
Third experimental week (4 weeks after hatching)	Fifth experimental week (6 weeks after hatching)
(II) Basal feed** of 16% protein	(VI) The feed same as (II)
(III) The feed (II) plus 4% additional glycine	(VII) The feed same as (III)
(IV) Basal feed*** of 20% protein	(VIII) The feed same as (IV)
(V) The feed (IV) plus 4% additional glycine	(IX) The feed same as (V)

* Specified in Table 1

** and *** Specified in Table 2

varying from 25 to 29°C during the periods of the experiment and the test chicks were allowed to take water and the feeds ad libitum. Body weight and feed consumption were recorded every 3 to 4 day period. Livers of 2 or 3 chicks of each group were removed after drainage of blood from carotid artery after 2 hr fasting and subsequent light anesthetization with ether. The removed livers were weighed and heated in test tubes containing boiling distilled water. Heating was continued for further 20 min in a boiling water bath. Time consumption for removal and weighing of the subjected liver was limited within 6 min to minimize the post-mortem change of the tissue. The boiled liver was treated by a homogenizer with deproteinizing agent of 0.6 N sulfuric acid and 10% sodium tungstate aqueous solution (7:5 in volume, mixed before use) according to the prescription by SCHURR *et al.*⁵⁾. The filtrate obtained from the deproteinized suspension thus obtained was adjusted to pH 6.8 with dilute sodium hydroxide solution and filled to an aliquot for amino acid assay. The microbiological

assay method using lactic acid bacteria was applied to the amino acid determination throughout the study. Liquid culture, titrating method on the uniform medium, was availably used for this purpose. *Streptococcus faecalis* R (ATCC 8043) was used as the test organism for threonine assay, *Leuconostoc citrovorum* 32 (ATCC 8081) for alanine and histidine and *Leuconostoc mesenteroides* P-60 (ATCC 8042) for other 15 amino acids.

Results and Discussion

As shown in table 4, weekly body weight gain and feed efficiency ($\frac{\text{weekly gain} \times 100}{\text{feeds amts. consumed}}$) showed a slight difference between 16% and 20% protein feed groups throughout the period of the feeding, the former being slightly inferior throughout the almost all stages of the growth. Wider depression of the weight gains was observed in glycine added feed groups throughout both the protein levels. But the difference in weekly gain was found to be wider at earlier stages of the growth (1st to 4th experimental weeks). The growth depression showed a tendency to be recovered at the later stage of the growth from the 5th experimental week, even not recognized significantly because of rather wider standard deviation of the figure of each week. No fatality of the test chicks was recorded throughout the experiment. The growth retardation and paralytic symptoms observed in the test chicks fed the glycine added feeds are assumed to be due to the known toxic nature of this amino acid.

Free amino acids of the livers are shown in table 5. In the observation at the 3rd experimental week, the values of glycine of groups III and V (specified in table 3) were

Table 4. Body weight gain (weekly) and the gain *per* feed consumed

Exptl. weeks	Weekly Body Weight Gain			
	Sixteen % protein		Twenty % protein	
	Basal	Basal + glycine	Basal	Basal + glycine
1st	60 ± 5	6 ± 17	68 ± 9	20 ± 4
2nd	84 ± 12	39 ± 12	81 ± 8	41 ± 3
3rd	76 ± 6	56 ± 11	89 ± 19	67 ± 8
4th	90 ± 14	65 ± 25	84 ± 3	75 ± 10
5th	70 ± 9	96 ± 8	119 ± 6	102 ± 2
(6th)	70 ± 8	75 ± 4	42 ± 18	55 ± 4
(7th)	58 ± 10	67 ± 8	69 ± 3	59 ± 2
	(Gain/Feed consumed) × 100			
1st	47	8	43	26
2nd	36	27	33	31
3rd	27	31	28	39
4th	24	23	19	25
5th	12	27	28	30
(6th)	16	18	8	14
(7th)	12	17	15	15

Figures of 6th and 7th experimental weeks were obtained from chicks having remained after amino acid assay experiment was completed (2 or 3 chicks).

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Table 5. Free amino acid values in the chick liver (γ /g raw basis)

Exptl. No.	(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)	(VIII)	(IX)
Time period and feeds	one week after hatching	Third experimental week				Fifth experimental week			
Amino acids		basal	basal+ glycine	basal	basal+ glycine	basal	basal+ glycine	basal	basal+ glycine
Glycine	71	67	350	59	240	180	180	110	140
Alanine	24	28	29	22	30	63	67	58	62
Arginine*	26	22	33	27	24	86	53	71	54
Aspartic acid	340	370	390	350	440	510	290	520	490
Cystine	—	7.6	15	12	6.7	24	17	18	17
Glutamic acid	340	380	550	410	490	740	660	690	640
Histidine*	14	47	21	19	22	57	74	63	49
Isoleucine*	24	23	22	26	19	46	38	50	33
Leucine*	59	43	48	60	49	80	76	80	84
Lysine*	30	49	64	68	43	74	62	120	77
Methionine*	25	29	44	33	32	29	42	37	33
Phenylalanine*	21	26	31	27	28	43	41	43	29
Proline	130	130	190	130	140	210	130	190	190
Serine	180	170	310	150	330	190	210	270	240
Threonine*	140	120	250	130	110	250	150	170	190
Tryptophan*	15	19	20	27	20	34	32	36	24
Tyrosine	18	19	19	15	24	39	37	43	30
Valine*	42	42	70	45	50	70	55	78	54
Total	1,499	1,592	2,456	1,610	2,098	2,725	2,214	2,647	2,436
Percentage of 10 essentials (asterisked) in total	26	26	25	29	19	28	28	28	26

evidently higher than those of the groups II and IV. The increase of glycine value (the values obtained from the glycine added group being subtracted by those from the none additional glycine group) were $3.7 \mu\text{mol}$ (16% protein feed) and $2.4 \mu\text{mol}$ (20% protein feed). The values of serine were also found to increase in the experiments III and V, the increases being computed as 1.3 and $1.7 \mu\text{mol}$ respectively in 16 and 20% protein feed groups. The total of the 18 free amino acids were approximately $1,500 \gamma/\text{g}$ in I, II and IV, and it surpassed $2,000 \gamma/\text{g}$ in other tests. No marked change assumed to be due to the addition of glycine was revealed in any of the amino acids assayed except those two mentioned above. In gross observation, the feature of the free amino acid was found to be reasonable when compared with those values in previous report on liver⁶⁾, characterized with higher glutamic acid, aspartic acid, proline and threonine valves and lower cystine value. Ten essentials in the 18 total were about 25 to 28% (with an exception in the experiment V). Although glycine and serine were observed to increase conspicuously in glycine added groups at 3rd experimental week, the 5th experimental subjects revealed increase neither of glycine nor of serine. For interpretation of the results, further experiments are necessitated.

Biochemical interconversion between glycine and serine was already well studied⁷⁾, but the reason of the discrepancy of the behavior of alteration of glycine and serine amount between

the observations in both the 3rd and 5th experimental weeks can not be explained by this experiment. Variety of glycine requirement during the experiment, change of tolerance through completion of liver function and domestication to this amino acid could be pointed out to understand the findings described here. B Vitamins, especially pyridoxine phosphate supplied to the test chicks are considered to fulfil the requirement enlisted in NRC standards, while the requirements possibly vary under the condition of inadequate balance of dietary amino acids. SWENDSEID *et al.*⁸⁾ demonstrated the relationships of these amino acids to which pyridoxine phosphate concerned. The relationships between growth of the test chicks and potencies of any amino acid in question in organ tissues as well as plasma should be necessarily studied in further works.

Summary

Test chicks were allowed to take the following feeds: One commercial chick feed for starting chick 1 week after hatching, two basal feeds containing 16 and 20% protein and two feeds of 4% glycine added to each of the basal feeds. Free amino acids of liver of the chicks thus maintained were determined by microbiological assay to which those of the standard feed, 4% glycine added feed groups were subjected. Among the free amino acid values of liver of the test chicks, free glycine and serine value in glycine added feeds in the 3rd week subjects were found to increase markedly. No other systematic alteration in the free amino acid values was observed throughout the study. Relationships among the growth gain, potencies of the amino acids in question and variations in the behavior of the amino acids in the week age were discussed.

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References

- 1) ALMQUIST, H.J. and E. MECCHI (1940) J. Biol. Chem., 135: 355—356.
- 2) ALMQUIST, H.J. and E. MECCHI (1942) Proc. Soc. Exptl. Biol. Med., 49: 541—543.
- 3) WIXOM, R.L., G.E. PIPKIN, J.H. WIKMAN and P.L. DAY (1958) J. Nutrition, 64: 13—31.
- 4) LONGENECKER, J.B. and N.L. HAUSE (1961) Am. J. Clin. Nutrition, 9: 356—362.
- 5) SCHURR, P.E., H.J. THOMPSON, L.M. HENDERSON and C.A. ELVEHJEM (1950) J. Biol. Chem., 182: 29—37.
- 6) SUGIMURA, K. and Y. GENDO (1965) Shokuryo Kenkyusho Hokoku, No. 19: 208—215.
- 7) SAKAMI, W. (1948) and (1949) J. Biol. Chem., 176: 995—996 and 178: 519—520.
- 8) SWENDSEID, M.E., J. VILLALOBOS and B. FRIEDRICH (1964) J. Nutrition, 82: 206—208.

単一アミノ酸過剰投与の幼雛器官遊離アミノ酸パターンに及ぼす影響

I. 過剰グリシン投与と幼雛肝の遊離アミノ酸

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動物の器官組織中のアミノ酸レベルは摂取タンパク質中のあるアミノ酸の過不足の判定に利用することが出来ると考えられるので、その一端を知る目的で実験を試みた。プリマスロック×ホワイトレグホーンの幼雛(雄性)を市販飼料で一週間飼育し、これより選出した幼雛に(1)基本配合飼料のタンパク質レベル 16% にしたもの、(2)同じくタンパク質レベル 20% にしたもの、(3)グリシン 4% を(1)に混じたもの、グリシン 4% を(2)に混じたもの、の 4 種類を群別に投与して飼育し、この食餌開始後第 3 週および第 5 週でそれぞれ肝の遊離アミノ酸 18 種を微生物法で測定した。全般に 16% タンパク

質の群は 20% のものよりわずかに成長が劣つたが、4% グリシンのものは更にそれぞれ著しく成育が遅滞した。しかし、週間成長量は第 5 週より回復の傾向を示した。肝中遊離アミノ酸のうちグリシンおよびゼリンが第 3 週の例においては、グリシン過剰投与群において著しく増量することが観察されたが、第 5 週の例では増量が認められなかった。その他のアミノ酸ではグリシン過剰投与によって系統的な増量または減量を示したものはなかった。これらの知見と幼雛の成長量との関連などに関して論述した。