

Interrelationship of Plasma Amino Acid Levels and Weight Gain in the Chick as Influenced by Suboptimal and Superoptimal Dietary Concentrations of Single Amino Acids

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ABSTRACT The concentration of either lysine, arginine or valine was varied over a wide range, in a basal diet containing crystalline amino acids in order to study the relationship between weight gain and the concentration of free amino acids in the blood plasma of chicks. It was shown that the first-limiting amino acid in the diet remains at a very low and constant level in the blood irrespective of the severity of the deficiency. Increments in excess of the amount needed to maximize weight gain resulted in a rapid and linear accumulation of that amino acid in the blood. Since the point at which the amino acid starts to accumulate in the plasma coincides with the break in the growth curve, it is concluded that the plasma technique can be used to determine the chick's requirement for amino acids.

Although assay procedures used in plasma amino acid studies have varied considerably, the results obtained have been quite consistent in demonstrating that dietary amino acid deficiencies result in reduced plasma concentration of that amino acid (1-3),^{1,2} whereas dietary amino acid excesses have resulted in accumulation of that amino acid in the plasma (4-7).³

However, it has not been possible to characterize the plasma amino acid response curve since in most instances only 2 dietary levels of an amino acid have been fed. The data of Morrison et al. (8) suggest that lysine does not accumulate in the plasma of growing rats when lysine is the growth-limiting amino acid in the diet. However, it has been demonstrated that plasma lysine accumulates in a linear manner when the concentration of dietary lysine is moderately in excess of that required to maximize growth in the turkey poul (6) and the growing rat (8), respectively.

It appears that when the growth of a young animal is restricted by an amino acid deficiency, the need of the tissues for that amino acid tends to maintain that nutrient at a minimal level in the blood. The present investigation was undertaken, therefore, to examine the effect of varying the dietary levels of single amino acids (lysine, arginine and valine) on the con-

centration of these amino acids in the blood plasma of growing chicks and to relate these observations to the growth-promoting ability of the experimental diets. Data were also obtained on the plasma pattern of amino acids other than the one varied in the diet.

EXPERIMENTAL

Male chicks, carefully selected from a large population for uniformity in initial weight after having been fed a pretest diet for seven or more days, were used in all experiments. Pertinent information concerning pretest diets, care and selection of experimental chicks has been described elsewhere (9).

The composition of the basal diet and the amino acid mixtures used are shown in tables 1 and 2, respectively. Except for methionine (DL-) only the L-isomer was used. Amino acid mixture A⁴ was used in

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¹ Dean, W. F. 1963 The development of a crystalline amino acid reference diet for chicks with special reference to its use in studying the effect of suboptimal and superoptimal dietary concentrations of amino acids on the free amino acid content of blood plasma. Doctoral Thesis, University of Illinois, Urbana.

² Smith, R. E. 1963 The utilization of the amino acids in intact proteins by the growing chick, with special reference to the effect of heating upon amino acid utilization, as measured by amino acid supplementation, biological (chick) assay of amino acids and free-amino acid patterns of blood plasma. Doctoral Thesis, University of Illinois, Urbana.

³ See footnote 1.

⁴ See footnote 1.

TABLE 1
Composition of basal diet

	% of diet
Cornstarch	variable
Amino acid mixtures	variable ¹
Corn oil, refined	15.00
Salt mixture ²	5.37
Cellulose ³	3.00
NaHCO ₃	1.00
Choline chloride	0.20
Vitamins ⁴	+
Total	100.00

¹ See experimental section and table 2.

² Mineral mixture, as per cent of the total diet: CaCO₃, 0.3; Ca₃(PO₄)₂, 2.8; K₂HPO₄, 0.9; MgSO₄·7H₂O, 0.35; ferric citrate, 0.05; ZnCO₃, 0.01; KI, 0.004; CuSO₄·5H₂O, 0.002; H₃BO₃, 0.0009; CoSO₄·7H₂O, 0.0001; MnSO₄·H₂O, 0.065; NaCl, 0.88; Na₂MoO₄·2H₂O, 0.0009; total 5.37.

³ Solka Floc, Brown Company, Chicago 3, Illinois.

⁴ G. J. Klain, H. M. Scott and B. C. Johnson, Poultry Sci., 39: 39, 1960.

TABLE 2
Composition of crystalline amino acid mixtures

Amino acid	Mixture A	Mixture B
	% contributed to the diet	
Arginine ¹	1.10	1.00
Histidine ¹	0.30	0.30
Lysine ¹	1.12	0.95
Tyrosine	0.63	0.45
Tryptophan	0.225	0.15
Phenylalanine	0.68	0.50
Methionine	0.55	0.35
Cystine	0.35	0.35
Threonine	0.65	0.65
Leucine	1.20	1.20
Isoleucine	0.80	0.80
Valine	0.82	0.82
Glycine	1.60	1.20
Proline	1.00	0.20
Glutamic acid	12.00	10.00
Total	23.025	18.92

¹ Supplied as arginine·HCl, histidine·HCl·H₂O and lysine·HCl.

experiment 5, and mixture B in experiments 2, 3, and 4. In experiments 1 and 6, mixture B, slightly modified to provide arginine, glycine and glutamic acid at the concentration indicated in mixture A was used. Access to feed and water was permitted at all times.

Blood samples were taken by heart puncture at the termination of the experiments immediately after obtaining final chick weights, using a syringe previously washed with a heparinized (0.6 mg/ml) 0.93% NaCl solution. The blood was then transferred to a heparinized (0.2 mg/ml blood) centrifuge tube and stored in an ice bath until it was centrifuged. In experiments 1,

5, and 6, plasma from 8 chicks was pooled, and plasma from 3 chicks was pooled in experiments 2, 3, and 4. After centrifuging the pooled plasma sample, a 10-ml aliquot was taken for analysis. The protein was precipitated with picric acid and further prepared for analysis as described by Stein and Moore (10). Two milliliters of the resultant 10-ml protein-free plasma were then added to the columns of a Beckman-Spinco Automatic Amino Acid Analyzer for amino acid analysis.

Where possible, the data for weight gain (gain/chick/day) and plasma amino acid (ug/ml) were analyzed statistically by the method of least squares.

RESULTS

The relationship of weight gain and plasma lysine to dietary lysine for chicks at different stages of growth¹ (exps. 1-4) are presented in tables 3 and 4. In the first experiment (table 3) the plasma lysine profile indicates that this amino acid did not accumulate in the plasma so long as the concentration of dietary lysine was less than that needed (0.83%) to maximize weight gain. Not until lysine levels in excess of 0.94% were fed did this amino acid start to accumulate rapidly in the plasma. This accumulation appears to proceed in a linear manner once the dietary requirement has been met.

In the older and larger chicks also, there was no suggestion that lysine would accumulate in the plasma when the concentration of dietary lysine was less than that needed to maximize weight gain (table 4). In general, lysine started to accumulate in the plasma at a rapid rate whenever the level of dietary lysine was approximately 10% in excess of that required to maximize weight gain.

Tables 5 and 6 record the results noted when the dietary concentration of valine and arginine were varied in a manner comparable to that described for lysine. In these instances also, the plasma pattern

¹ The experimental diets were fed over the period of 8 to 15 days (70), 15 to 21 days (157), 22 to 28 days (278) and 29 to 33 days (424) in experiments 1, 2, 3 and 4, respectively. The numbers in parentheses represent the average chick weight (g) of all experimental groups at the start of each assay. In both experiments 5 (valine assay) and 6 (arginine assay) the experimental diets were fed for 7 days extending from the 8th to the 15th day. The initial starting weights were 73 and 77 g for experiments 5 and 6, respectively.

TABLE 3
Effect of feeding graded levels of lysine on plasma amino acids and weight gain (exp. 1)

Amino acid	% Dietary lysine							
	0.60	0.70	0.80	0.90	1.00	1.20	1.40	1.80
	<i>μg of amino acid/ml plasma</i>							
Threonine	141.4	192.1	108.8	82.8	47.6	37.0	44.8	126.1
Proline	25.2	24.4	22.0	22.4	25.8	22.2	22.0	19.5
Glutamic acid	44.4	58.4	44.3	66.2	73.5	32.3	42.8	27.7
Glycine	108.0	97.1	73.8	84.1	125.1	60.4	78.1	64.8
Valine	31.9	31.0	29.3	20.4	16.9	15.2	15.1	22.7
Cystine	21.2	—	32.7	25.6	—	30.4	26.4	33.2
Methionine	17.2	17.4	15.5	14.2	13.5	10.6	12.9	12.4
Isoleucine	17.1	14.6	13.5	9.2	8.8	8.2	9.8	12.0
Leucine	21.3	21.3	17.2	14.6	12.6	10.7	13.1	14.6
Tyrosine	23.9	17.3	13.9	9.7	5.4	—	4.7	11.2
Phenylalanine	15.5	14.5	12.4	9.1	4.9	—	4.7	8.4
Histidine	21.2	15.9	10.4	5.0	4.1	1.7	3.4	10.2
Arginine	66.0	69.7	59.5	61.8	45.2	25.0	25.3	6.1
Lysine	6.8	9.7	7.2	15.5	23.1	65.7	106.7	233.7
Gain/chick/ day, g ¹	8.2	11.1	12.2	13.6	12.8	12.9	13.2	10.7
Gain/feed	0.57	0.64	0.68	0.74	0.75	0.77	0.73	0.64

¹ Average of duplicate groups of 5 chicks/treatment. See footnote 5 in text for data concerning initial starting weight and duration of assay.

TABLE 4
Effect of feeding graded levels of lysine on plasma lysine and weight gain

Dietary lysine	Experiment 2		Experiment 3		Experiment 4	
	Plasma lysine	Gain/chick/day ¹	Plasma lysine	Gain/chick/day ¹	Plasma lysine	Gain/chick/day ¹
	%	<i>μg/ml</i>	<i>g</i>	<i>μg/ml</i>	<i>g</i>	<i>μg/ml</i>
0.4	7.5	6.3	5.4	10.8	5.7	15.5
0.5	9.8	10.5	4.8	15.8	4.7	20.5
0.6	7.8	14.5	4.4	23.0	6.2	29.3
0.7	6.2	19.8	9.9	26.8	20.4	29.0
0.8	9.4	19.8	18.2	27.2	15.9	29.5
0.9	27.8	19.2	44.4	27.0	26.5	28.0
1.0	—	—	—	—	62.4	32.5

¹ Average of triplicate groups of 3 chicks/treatment. See footnote 5 in text for data concerning initial starting weight and duration of assay.

for valine and arginine reflect the same basic characteristics as observed for lysine.

DISCUSSION

From this series of experiments, it has been shown that there is a definite relationship between plasma amino acid levels and the amino acid adequacy of the diet. The first-limiting amino acid remains at a very low level in the blood irrespective of the severity of the amino acid deficiency ("imbalance"). This observation helps to explain why in some instances, supplementing diets with the first-limiting amino acid has failed to increase the concentra-

tion of this amino acid in the plasma (4, 11). According to observations noted herein, no increase would be anticipated until the dietary level exceeded that needed to maximize growth.

The presence of an amino acid in the diet, in excess of that required to maximize weight gain, consistently resulted in a rapid accumulation of that amino acid in the plasma. This accumulation appears to proceed in a linear manner, even though, as was the case for lysine (table 3) and valine (table 5), the greatest dietary concentration used was more than twice that required to maximize weight gain. More-

TABLE 5
Effect of feeding graded levels of valine on plasma amino acids and weight gain (exp. 5)

Amino acid	% dietary valine							
	0.57	0.67	0.72	0.77	0.82	0.87	1.07	1.82
	<i>μg of amino acid/ml plasma</i>							
Threonine	78.2	65.0	46.0	35.2	36.0	40.6	27.3	49.5
Proline	40.5	46.2	36.5	38.4	34.1	44.7	30.3	48.7
Glutamic acid	35.6	36.4	36.2	43.4	39.7	44.7	22.0	42.6
Glycine	88.0	93.9	75.8	73.8	56.7	94.8	44.4	76.2
Cystine	25.3	27.0	33.0	32.2	34.5	29.7	44.6	29.6
Methionine	15.3	18.0	16.6	13.9	13.4	18.9	14.7	18.7
Isoleucine	14.2	13.9	10.8	10.3	7.4	10.7	8.0	9.5
Leucine	21.7	17.3	13.5	13.7	10.6	12.9	9.8	10.7
Tyrosine	30.6	25.8	24.4	25.9	21.1	27.1	18.7	21.0
Phenylalanine	16.6	12.6	10.3	11.0	9.1	10.6	8.9	8.2
Lysine	39.9	60.7	46.4	40.5	34.3	24.5	19.0	65.0
Histidine	3.8	5.6	2.2	2.4	0.4	0.6	nd ¹	4.3
Arginine	—	38.9	23.8	21.2	16.5	—	19.1	26.8
Valine	6.3	9.1	6.3	10.7	10.6	19.2	35.7	127.7
Gain/chick/day, g ²	6.6	11.1	12.4	13.7	14.1	13.3	13.9	13.6
Gain/feed	0.57	0.72	0.76	0.78	0.79	0.76	0.78	0.76

¹ None detectable.

² Average of duplicate groups of 5 chicks/treatment. See footnote 5 in text for data concerning initial starting weight and duration of assay.

TABLE 6
Effect of feeding graded levels of arginine on plasma amino acids and weight gain (exp. 6)

Amino acid	% dietary arginine					
	0.60	0.70	0.80	0.90	1.00	1.10
	<i>μg of amino acid/ml plasma</i>					
Threonine	217.7	164.7	123.1	79.5	81.4	65.6
Proline	30.8	32.0	24.0	26.5	23.7	25.6
Glutamic acid	55.1	102.4	103.1	117.5	87.6	117.4
Glycine	98.1	86.3	134.3	128.3	125.3	86.9
Valine	39.8	35.7	28.6	24.1	28.7	22.6
Cystine	38.5	22.1	21.5	19.9	15.4	20.5
Methionine	16.6	13.4	17.6	14.2	17.0	13.3
Isoleucine	23.3	22.9	19.1	14.7	18.0	13.9
Leucine	31.9	28.7	21.8	19.3	23.0	18.5
Tyrosine	—	20.8	14.2	9.7	10.3	8.0
Phenylalanine	—	15.5	12.0	10.7	12.1	9.2
Lysine	109.2	97.0	65.4	39.3	37.5	30.8
Histidine	21.7	20.0	14.1	5.7	6.3	5.8
Arginine	4.8	7.9	23.1	11.4	39.5	46.0
Gain/chick/day, g ¹	9.9	11.3	12.4	13.6	13.8	13.8
Gain/feed	0.54	0.59	0.65	0.69	0.72	0.72

¹ Average of triplicate groups of 5 chicks/treatment. See footnote 5 in text for data concerning initial starting weight and duration of assay.

over, in the case of lysine, this amino acid continued to accumulate linearly in the plasma, even though growth was depressed 20% by the highest (1.8%) level fed. Exceedingly high plasma amino acid levels have been reported by others (5)⁶ under similar conditions.

That the shape of the plasma amino acid curve can be used to determine the amino

acid requirement of the chick has been demonstrated in these studies. More recently, Mitchell⁷ has reported good agreement between data acquired by the plasma technique and nitrogen balance in deter-

⁶ See footnote 1.

⁷ Mitchell, J. R., Jr. 1965 Effect of level of amino acid intake on the nitrogen metabolism and plasma free amino acids of the young pig. Doctoral Thesis, University of Illinois, Urbana.

mining the young pig's requirement for several amino acids.

From the data obtained in this series of experiments, it was possible to calculate the chick's requirement for lysine at successive stages of development. Analysis of the data by the method of least squares indicates that the lysine requirement, expressed as a percentage of the diet, was 0.83, 0.70, 0.67 and 0.59% for the second, third, fourth and fifth week of life, respectively.

In experiments 1, 5 and 6, plasma samples were analyzed for both essential and non-essential amino acids (tryptophan excepted) but only data concerning the amino acids provided in the experimental diets are presented in the tabulations. In experiments 2, 3 and 4 the plasma was analyzed for lysine only. Severe deficiencies of either lysine or arginine markedly increased plasma threonine. Plasma threonine varied from a high of approximately 190 to a low of 40 $\mu\text{g}/\text{ml}$ in the lysine assay (table 3) and from 217 to 65 $\mu\text{g}/\text{ml}$ in the arginine experiment (table 6). Each increment of the first-limiting amino acid (lysine or arginine) resulted in a progressive decline in plasma threonine. However, the point where plasma threonine reaches its lowest concentration does not coincide with the dietary concentration of the first-limiting amino acid that maximizes weight gain, but rather at a level somewhat in excess of that needed for most rapid weight gain. However, this does not appear to be unique for threonine since many of the other amino acids also behave in this manner although obviously to a lesser degree. It follows also that the rapid decline in plasma threonine concentration should not be interpreted as indicating that it is the next limiting amino acid. Large excesses of dietary lysine or valine also increased the concentration of plasma threonine. Others (2, 3, 7)^{*} have shown that amino acid imbalances will influence the concentration of threonine in the plasma. Lysine also appears to accumulate in the plasma to a marked degree when the diet is deficient in arginine (table 6). There is a suggestion that plasma arginine tends to accumulate in the plasma when the diet contains suboptimal amounts of either lysine or valine. As for the other

amino acids, it appears that histidine, tyrosine, phenylalanine, leucine and isoleucine tend to accumulate in the plasma to a slight degree irrespective of whether lysine, arginine or valine were deficient whereas proline, glutamic acid, glycine, methionine and cystine were, for the most part, either unaffected by the treatments imposed, or were so variable as to preclude the establishment of a definite plasma pattern. Dean,⁹ however, has reported that all amino acids (except lysine) tend to accumulate in the plasma when chicks are fed diets grossly deficient in lysine. In general, the observations noted herein support the view that diets containing well balanced protein would tend to minimize the accumulation of free amino acid nitrogen in blood plasma.

In experiment 1 where the greatest amount of lysine fed (1.8%) exceeded the requirement level approximately twofold, several amino acids tended to accumulate in the plasma. A notable exception was arginine, whose concentration continued to decrease (table 3), and the possibility should be considered whether this observation is in some way related to the demonstrated ability of arginine to reverse the growth-depressing effect of excess dietary lysine (12). Furthermore, the data presented herein appear to indicate that variations in the concentration of dietary lysine or arginine exert a more pronounced effect on the plasma concentration of other amino acids than does valine which may reflect the difference in "relative toxicity" among amino acids for the chick (13).

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^{*} See footnote 1.

⁹ See footnote 1.

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