Effect of Dietary Amino Acid Level, Folic Acid, Glycine, and Serine on Chick Performance and Blood Parameters[1]

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ABSTRACT Studies of the interrelationships between dietary amino acid level, folic acid, glycine, and serine in chicks fed crystalline amino acid diets indicated that a folic acid deficiency exerted a greater detrimental effect on chick growth than did a deficiency of glycine and serine. The increased chick growth observed when a folic acid deficient diet containing normal levels of amino acids was supplemented with glycine and serine suggested that these amino acids were exerting a folic acid sparing effect. A similar sparing effect was not noted when the diet contained a high level of dietary amino acids which resulted in increased uric acid synthesis. Blood hemoglobin levels appeared to be influenced by a combination of factors including dietary folic acid and glycine levels and possibly growth rate of the chicks. Plasma glycine and serine concentrations were closely associated with the presence or absence of these amino acids in the diet and the level of the other amino acids in the diet. Within groups receiving the same level of dietary glycine and serine, lower plasma glycine was associated with higher levels of plasma uric acid.

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INTRODUCTION

It is well established that glycine synthesis by the chick is not adequate to support maximal growth. Studies indicate that chicks fed a glycine and serine free crystalline amino acid diet grow at approximately 75 to 80% the rate of chicks fed the same diet supplemented with glycine (Baker *et al.*, 1968, 1972; Baker and Sugahara, 1970; Featherston, 1975). An equimolar quantity of L-serine has been shown to support equal chick performance as glycine, thus indicating that serine conversion to glycine is adequate to meet the dietary need for glycine (Akrabawi and Kratzer, 1968; Baker *et al.*, 1968; Rabbani *et al.*, 1973; Featherston, 1975).

Folic acid is closely associated with glycine and serine utilization in that the folic acid-containing enzyme serine hydroxymethyl transferase is responsible for the interconversion of these two amino acids. Rabbani *et al.* (1973) showed that L-serine was not as effective as glycine for chick growth when added to a glycine-serine-free diet that was deficient in folic acid. They observed the activity of serine hydroxymethyl transferase to decrease in the presence of a folic acid deficiency. Folic acid is also involved in purine and uric acid production at two steps in the synthetic pathway.

High dietary levels of glycine, conversely, may increase the deleterious effects of a folic acid deficiency. Kratzer and Lantz (1957) found that glycine supplementation of poult diets exaggerated a folic acid deficiency and caused depressed growth, increased mortality, and increased incidence of cervical paralysis. In studies of the effect of diet on folic acid requirements in chicks, Wong *et al.* (1977) observed the most pronounced effects of a folic acid deficiency as measured by low hematocrit values and the incidence of leg abnormalities with a casein-gelatin diet (supplemented with DL-methionine and L-arginine) which contained 2.05% glycine.

Studies were conducted to determine the interrelationship of dietary folic acid, glycine, and serine in the young chick fed crystalline amino acid diets. The other dietary amino acids were fed at one and three times the requirement level to stimulate different rates of uric acid synthesis.

MATERIALS AND METHODS

The folic acid deficient basal diet used in these studies is shown in Table 1. L-glutamic acid was added to the diets not receiving glycine and/or serine so that all diets were isonitrogenous. Three times the levels of amino acids shown in Table 1 were added at the

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expense of corn starch in some diets in Experiments 2 and 3.

Day-old White Mountain cockerels were reared in electrically heated batteries with raised wire floors. Four pens with 5 chicks each were fed a randomly assigned experimental diet for a 14-day experimental period.

At the end of each experiment, 8 chicks per treatment (2 per replicate) were bled for analysis of blood hemoglobin and plasma uric acid, glycine and serine concentrations. The total hemoglobins of blood were determined as alkaline hematin by the procedure of Bell *et al.* (1965). Plasma uric acid was determined using the uricase method of Blauch and Koch (1939) as described in a technical bulletin[2]. Hemoglobin and uric acid determinations were conducted on individual samples. Plasma for amino acid analysis was deproteinized by adding sulfosalycilic acid to plasma to give a final concentration of 3% sulfosalycilic acid. Plasma glycine and serine were determined by use of an amino acid analyzer[3]. Pooled samples for each replicate (4 samples) were analyzed for glycine and serine in Experiment 2, whereas in Experiment 3 only 1 pooled sample representing 2 chicks from each of 4 replicates was analyzed.

Data were analyzed statistically by analysis of variance. Treatment means were tested for significant differences by the sequential method of Newman and Keuls (Steel and Torrie, 1960).

TABLE 1. *Composition of folic acid deficient basal diet*

Ingredient	(%)
Corn starch	47.66
Corn oil	15.00
Vitamin and mineral premix[a]	13.50
Sodium bicarbonate	1.00
L-arginine•HCl	1.15
L-histidine•HCl•H_2O	.41
L-lysine•HCl	1.14
L-tyrosine	.45
L-phenylalanine	.50
L-tryptophan	.15
DL-methionine	.35
L-cystine	.35
L-threonine	.65
L-leucine	1.00
L-isoleucine	.60
L-valine	.69
L-proline	.40
L-glutamic acid	10.00
Variables[b]	5.00

[a]The vitamin and mineral premix provided the following ingredients in g/kg of diet: minerals, 60.882; vitamins, 20; cellulose, 30; choline chloride, 2; butylated hydroxy toluene, .125; glucose monohydrate, 22. The mineral premix provided the following reagent minerals in g/kg of diet: $CaCO_3$, 19.1; $Ca(H_2PO_4)_2$•$2H_2O$, 21.15; K_2HPO_4, 11.2; NaCl, 6.0; $MgCO_3$, 2.08; $FeSO_4$, .2; $ZnCO_3$, .18; $CuSO_4$•$5H_2O$, .015; $MnSO_4$•H_2O, .51; KI, .04; $NaMoO_4$•$2H_2O$, .0025; and Na_2Se_3O, .00022. The vitamin premix provided the following units of vitamins per kg of diet (in mg): thiamine•HCl, 6.0; riboflavin, 9.0; niacin, 50.0; Ca d-pantothenate, 20.0; pyridoxine•HCl, 8.0; biotin, .3; menadione sodium bisulfite, 2.0; inositol, 1000.0; and vitamin B_{12}, 20 μg; vitamin A palmitate, 25,000 USP units; vitamin D_3, 1200 ICU; d-α-tocopheryl acetate, 17.6 IU.

[b]Additions of folic acid, glycine, L-serine, L-glutamic acid, and corn starch to total 5.0% of the diet.

RESULTS AND DISCUSSION

Experiment 1 was designed to determine whether serine was as effective as glycine in supporting chick growth when added in equimolar amounts to a crystalline amino acid diet without or with folic acid supplementation at a level of 11 mg/kg of diet. In comparing the weight gain of chicks fed diets supplemented with folic acid, an equal response was noted from the addition of equimolar amounts of serine or glycine (Table 2). The response to supplemental glycine or serine was of a similar magnitude, approximately 20%, as that observed in previous studies (Featherston, 1975).

A dietary deficiency of folic acid resulted in markedly lower weight gains. Adding serine to the folic acid deficient diet had little effect whereas adding glycine gave a significant ($P<.05$) improvement in weight gain and feed efficiency. The results are in agreement with previous studies by Rabbani *et al.* (1973) who observed that serine was not as effective as glycine when the diet was deficient in folic acid, presumably because of a decrease in the activity of serine hydroxymethyl transferase needed for the conversion of serine to glycine.

Blood hemoglobin levels appeared to be influenced by a combination of factors including dietary folic acid and glycine levels and possibly growth rate of the chicks. With Diets 1

[2]Technical Bulletin No. 680, 1965, Sigma Chemical Company, St. Louis, MO.
[3]Technicon Instruments Corporation, Ardsley, NY 10502.

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TABLE 2. *Effect of dietary supplementations of glycine, serine, and folic acid on chick performance and blood parameters (Experiment 1)*

Diet no.	Dietary variable: Glycine or serine	Dietary variable: Folic acid	Weight[1] gain	Feed/ gain	Blood hemoglobin	Plasma uric acid
	(%)	(mg/kg)	(g)		(g/100 ml)	(mg/100 ml)
1	...	...	83.7^{d}	1.97^{a}	11.22^{a}	3.62^{a}
2	...	11	156.0^{b}	1.51^{b}	9.33^{b}	4.29^{a}
3	1.68 serine	...	90.1^{d}	1.81^{a}	11.00^{a}	3.24^{a}
4	1.68 serine	11	188.8^{a}	1.44^{b}	9.50^{b}	3.77^{a}
5	1.20 glycine	...	130.6^{c}	1.48^{b}	4.14^{c}	3.97^{a}
6	1.20 glycine	11	187.2^{a}	1.42^{b}	10.53^{ab}	4.19^{a}

^{a,b,c,d}Means for a given parameter with the same lettered superscript within a column are not significantly different (P>.05). L-serine (1.68%) and glycine (1.20%) were compared on an equimolar basis.

[1] Average daily gain of 4 pens of 5 chicks each from 0 to 14 days of age.

and 3, where growth rate was poor on these folic acid deficient diets, blood hemoglobin levels were high. When glycine was added to the folic acid deficient diet (Diet 5) and more rapid growth occurred, a low blood hemoglobin resulted. Similar results were noted in all three experiments reported herein. No significant (P>.05) differences were noted in plasma uric acid between any of the different groups.

In Experiment 2, three factors were investigated: 1) two levels of dietary amino acids, one and three times the requirement; 2) 0 and 1.20% glycine plus 1.68% L-serine supplementation; and 3) 0 and 11 mg folic acid/kg of diet (Table 3). Since folic acid is a cofactor of serine hydroxymethyl transferase, this experiment was designed to see if a glycine deficiency would be more severe with chicks fed a folic acid deficient, glycine and serine free diet as compared with chicks fed the same diet with added folic acid. The two levels of dietary amino acids were superimposed on the above design to attempt to force two different rates of uric acid synthesis.

Poor chick growth was observed with the double deficient diet containing normal levels of amino acids (Diet 1). Supplementation of the diet with either folic acid or glycine plus serine resulted in a marked improvement in chick growth with the best performance being observed with the diet supplemented with folic acid, glycine, and serine. A similar trend was observed with chicks fed high levels of amino acids with the exception that, in contrast to the normal level amino acid series, the glycine plus serine supplementation was totally ineffective in overcoming the effects of the folic acid deficiency on chick growth. A similar pattern of weight gain was noted in Experiment 3 (Table 4). Possibly the glycine plus serine supplementation was sparing folic acid with the one times level of amino acids, but at the three times level of amino acids the demand for folic acid for a high rate of uric acid synthesis may have been so great that the sparing action of glycine plus serine was inadequate.

The small chicks fed the folic acid deficient diet (Diet 1) did not show abnormal hemoglobin values (Table 3). When more rapid growth was stimulated in folic acid deficient chicks by supplementing with glycine plus serine, as in Diet 3, significantly lower blood hemoglobin values resulted. At the high level of amino acids, the hemoglobin values of all groups were lower, possibly due to lower feed intake per gram weight gain, hence lower intake of all nutrients except amino acids, than was noted with the one times series. Lower hemoglobin values were noted in both treatments of folic acid deficient chicks consuming high levels of amino acids than those observed with the chicks receiving folic acid. Chicks fed Diet 7, which was supplemented with glycine plus serine, exhibited lower values than those of chicks fed the folic acid deficient diet without glycine and serine additions, however.

No significant differences were noted in plasma uric acid values for any of the chicks fed the normal level of amino acids. All treatment groups of chicks fed the high level of amino

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TABLE 3. *Effect of dietary amino acid level, folic acid, glycine and serine on chick performance and blood parameters (Experiment 2)*[1]

Diet no.	Dietary amino acids	Dietary variable: Glycine	Dietary variable: Serine	Dietary variable: Folic acid	Weight gain	Feed/ gain	Blood hemoglobin	Plasma uric acid	Plasma glycine	Plasma serine
		(%)	(%)	(mg/kg)	(g)		(g/100 ml)	(mg/100 ml)	(mg/100 ml)	(mg/100 ml)
1	1X	...	...	...	65.7[c]	2.31[a]	11.95[a]	2.89[c]	1.51[d]	14.47[b]
2	1X	...	...	11	128.8[b]	2.03[ab]	10.40[ab]	4.14[c]	1.56[d]	7.33[b]
3	1X	1.20	1.68	...	120.0[b]	1.60[b]	6.35[de]	3.55[c]	24.63[a]	50.73[a]
4	1X	1.20	1.68	11	148.4[a]	1.74[b]	10.77[ab]	3.00[c]	21.17[b]	46.28[a]
5	3X	...	...	...	58.4[c]	1.68[b]	7.55[cd]	6.63[b]	5.18[d]	11.25[b]
6	3X	...	...	11	126.7[b]	1.27[c]	9.08[bc]	11.18[a]	1.51[d]	5.57[b]
7	3X	1.20	1.68	...	58.5[c]	1.64[b]	5.10[e]	6.96[b]	13.60[c]	13.10[b]
8	3X	1.20	1.68	11	153.4[a]	1.08[c]	9.74[b]	11.67[a]	5.38[d]	11.28[b]

[a,b,c,d,e] See Table 2 footnote.

[1] See Table 2 footnote.

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TABLE 4. *Effect of dietary amino acid level, folic acid, glycine and serine on chick performance and blood parameters (Experiment 3)*[1]

Diet no.	Dietary amino acids	Glycine	Serine	Folic acid	Weight gain	Feed/ gain	Blood hemoglobin	Plasma uric acid	Plasma glycine	Plasma serine
		(%)		(mg/kg)	(g)		(g/100 ml)	(mg/100 ml)		
1	1X	...	...	...	82.6[e]	1.81[a]	9.42[a]	5.29[c]	2.27	11.46
2	1X	...	...	11	155.2[c]	1.50[bc]	8.76[a]	5.57[c]	2.13	4.40
3	1X	1.20	1.68	...	153.7[c]	1.41[c]	5.06[b]	5.11[c]	24.64	37.76
4	1X	1.20	1.68	11	210.0[a]	1.32[c]	9.12[a]	4.23[c]	22.58	31.59
5	3X	...	...	...	66.2[f]	1.40[c]	5.63[b]	7.69[b]	5.37	5.96
6	3X	...	...	11	131.1[d]	1.15[d]	8.14[a]	12.87[a]	1.94	4.23
7	3X	1.20	1.68	...	63.6[f]	1.49[bc]	4.55[b]	9.32[b]	8.45	7.85
8	3X	1.20	1.68	11	176.4[b]	1.07[d]	8.81[a]	12.39[a]	4.57	8.16
9	1X	...	3.36	...	87.9[e]	1.61[b]	8.49[a]	3.66[c]	2.22	109.16
10	3X	...	3.36	...	77.5[e]	1.36[c]	5.90[b]	8.96[b]	8.31	13.98

[a,b,c,d,e,f] See Table 2 footnote.

[1] See Table 2 footnote.

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acids had elevated plasma uric acid values as compared to the normal amino acid series. Chicks fed the diets containing folic acid had significantly higher plasma uric acid values than folic acid deficient chicks. A possible explanation for these results is that the folic acid adequate chicks had a greater capacity to synthesize uric acid which permitted a more rapid breakdown of the excess dietary amino acids, with excretion of the nitrogen and utilization of the carbon skeleton for energy. Plasma uric acid concentration has been observed to be highly correlated with total uric acid excretion (r = .96)[4].

The plasma concentrations of glycine and serine were closely related to the dietary treatments. Lower levels of plasma glycine and serine resulted from dietary supplementation of the three times amino acid diets with glycine and serine than occurred when the one times amino acid diets were similarly supplemented. This probably is an indication of a higher amino acid catabolic capacity of the chicks adapted to the high amino acid diets and the fact that higher levels of all amino acids were being consumed than occurred with the one times amino acid diets.

The third experiment was identical to Experiment 2 with the exception that two extra diets were added in which the glycine in the glycine plus serine supplementation was replaced by an equimolar amount of additional serine (Table 4). Similar growth responses were noted in this experiment as were observed in Experiment 2. With the one times level of amino acids, a marked growth response to either folic acid or glycine plus serine supplementation was observed with the best growth occurring when both were added. With the three times level of amino acids, a response to folic acid but no response to glycine plus serine was noted. As in Experiment 1, serine alone was ineffective in countering the effects of a folic acid deficiency.

The blood hemoglobin values demonstrated a similar pattern as in Experiment 2: normal hemoglobin levels in the small chicks fed Diet 1 but significantly lower hemoglobin values when growth was stimulated in folic acid deficient chicks by glycine plus serine supplementation (Diet 3). With the three times level of amino acids, both groups fed folic acid deficient diets had lower blood hemoglobin, as was the case in Experiment 2. The folic acid deficient treatments supplemented with serine alone showed generally similar growth and hemoglobin values to the negative controls for the one times and three times levels of amino acids, respectively.

The present studies with chicks fed normal levels of amino acids do not distinguish whether the lowered blood hemoglobin results from a direct metabolic interaction with the glycine added to the diets or the indirect result of the more rapid growth accentuating the existing folic acid deficiency. The lowered hemoglobin values without differences in growth rate when glycine and serine were added to the high amino acid diets, however, would suggest that this effect is directly attributable to the glycine and serine supplementation. Wong *et al.* (1977) suggested that high dietary glycine may produce anemia by increasing the folic acid requirement of chicks.

Plasma uric acid, glycine, and serine values showed similar trends as those noted in Experiment 2. The lower plasma glycine values noted in Treatments 6 and 8 were associated with higher plasma uric acid levels as compared with Treatments 5 and 7, respectively, which did not receive folic acid supplementation. These results are consistent with the suggestion that uric acid synthesis is being limited by the folic acid deficiency in chicks fed the high levels of amino acids. The lack of an effect of serine supplementation of the diet containing the normal level of amino acids (Diet 9) on plasma glycine concentration as opposed to the markedly elevated levels of plasma serine agrees with the previous work of several investigators (Sugahara and Ariyoshi, 1967; Akrabawi and Kratzer, 1969; Coon *et al.*, 1974). The observation that serine supplementation of the diet containing the high level of amino acids resulted in higher plasma glycine and lower plasma serine than occurred with a similar dietary level of serine in the normal amino acid diet may indicate an increased enzymatic capacity to convert serine to glycine as well as increased serine catabolism.

[4] Unpublished data, W. R. Featherston and R. D. Miles.

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