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Uric Acid Excretion as an Indicator of the Amino Acid Requirement of Chicks[1] (37875)

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Numerous methods have been developed for determinations of amino acid requirements and quality of dietary proteins. These range from assays which measure only growth to those which relate amino acid requirement to polyribosome profiles in liver cells. Determinations of biological value which involve the analysis of fecal and urinary nitrogen excretion are difficult in birds because of the problems associated with separate collections of feces and urine. Uric acid is the primary nitrogenous excretory product in birds. If it is assumed that all the uric acid present in excreta is of urinary origin, the measurement of uric acid concentration might serve as a useful index of nitrogen catabolism without requiring urine and fecal separation.

Kiriyama and Iwao (1) measured urea excretion of rats fed diets containing threonine levels ranging from suboptimal to supraoptimal. When urinary urea nitrogen per gram of ingested nitrogen was plotted against the threonine level in the diet, a linear decrease was noted until the requirement level for threonine was reached. At that point, the ratio plateaued or increased. Brown and Cline (2) noted a linear decrease in urinary urea excretion by pigs fed a corn-based diet which was supplemented with 0.05% DL-tryptophan and increasing levels of L-lysine ranging from 0 to 0.45% of the diet.

The present study was conducted to investigate uric acid excretion in the chick fed diets containing graded quantities of a limiting amino acid and to compare this parameter with weight gain as an indicator of the amino acid requirement. Plasma uric acid concentrations and liver xanthine dehydrogenase activities were also determined in one experiment.

Materials and Methods. Day-old, male, broiler-type chicks were fed a diet containing 25% isolated soybean protein (3) for a pre-experimental period of 7–10 days. At this time, the chicks were randomly assigned to pens of 5 chicks each, two pens per dietary treatment. In Expt 1, a crystalline L-amino acid basal diet was patterned after that of Velu *et al.* (4) with the exception that the vitamin and mineral premix described by Scholz and Featherston (3) was used. The basal diet contained 0.5 L-lysine and was supplemented with L-lysine·HCl to provide 8 diets with lysine contents ranging from 0.5 to 1.2% at 0.1% increments. L-lysine·HCl was added on an isonitrogenous basis at the expense of L-glutamic acid.

In Expts 2 and 3, the glucose–safflower meal diet contained (in %): safflower meal, 48.0; glucose monohydrate, 32.3; vitamin and mineral premix (3); 12.5; corn oil, 5.0; DL-methionine, 0.2; and variables, 2.0. The 21.3% protein diet was analyzed to contain 0.62% lysine. The basal diet was supplemented with L-lysine·HCl to provide 8 diets with lysine contents ranging from 0.62 to 1.32% at 0.1% increments. L-Glutamic acid was added in varying quantities to maintain all diets at the same

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nitrogen level.

The diets and water were offered *ad lib.* during the 7-day experimental period from 7 to 14 days of age in Expts 1 and 3 and 10–17 days of age in Expt 2. Feed consumption on a per pen basis was recorded daily. Excreta were collected for uric acid analysis during the last 2 days of the experimental period. Excreta were collected in 0.5% lithum carbonate solution and the uric acid content determined as described previously (5).

In Expt 3, blood samples were collected at the end of the experiment by cardiac puncture using heparinized needles and syringes. Plasma uric acid concentration was determined by the uricase method as described in a technical bulletin.[2] Livers were also removed from the chicks in this experiment and the xanthine dehydrogenase activity was measured spectrophotometrically at 340 nm as the rate of formation of NADH. Assay procedures were identical to those reported previously (3).

The least-squares method of analysis was used to analyze weight gain and uric acid excretion data from the 3 experiments (6). Plasma uric acid and xanthine dehydrogenase activity data were analyzed statistically by analysis of variance. Treatment means were tested for significant differences by the sequential method of Newman and Keuls (6).

Results and Discussion. Feed consumption of chicks fed diets containing the lower levels of lysine was decreased compared with that of chicks fed diets containing higher levels of lysine. Uric acid excretion was therefore expressed as grams uric acid excreted per day per gram nitrogen consumed. Even when expressed on the basis of total nitrogen consumed, chicks fed the diets containing inadequate lysine excreted more uric acid, indicating that more of the dietary amino acids were being catabolized due to insufficient dietary lysine for maximal protein synthesis.

Uric acid excretion decreased and weight gain increased in a linear fashion as the lysine content of the diet was increased to the chick's requirement (Figs. 1–3). At this point, uric acid excretion and weight gain plateaued with only slight changes observed at the higher dietary levels of lysine. In Expt 1, analysis of the weight gain data from chicks fed the crystalline L-amino acid diets indicated a lysine requirement of 0.88%, whereas with uric acid excretion

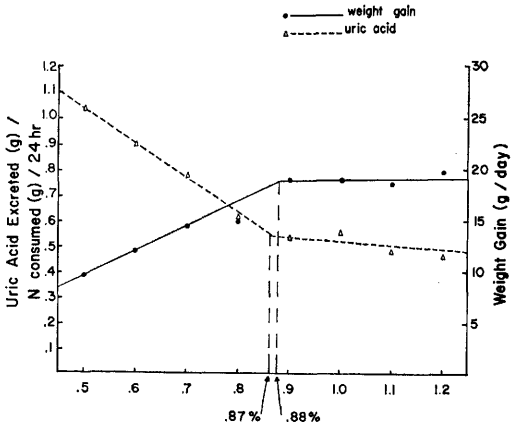

FIG. 1. Weight gain and uric acid excretion of chicks fed crystalline L-amino acid diets with lysine contents ranging from 0.5 to 1.2% from 7 to 14 days of age (Expt 1).

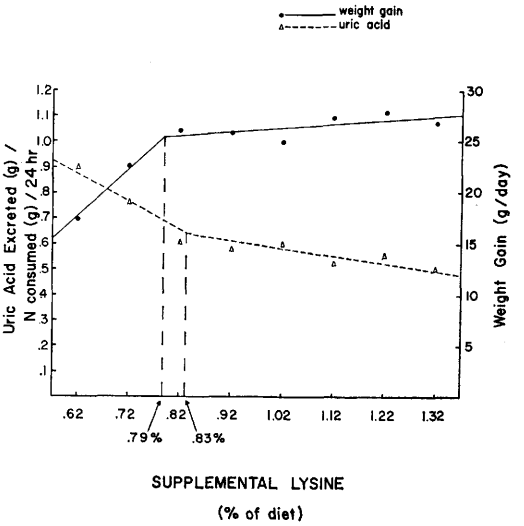

FIG. 2. Weight gain and uric acid excretion of chicks fed glucose–safflower meal diets with lysine contents ranging from 0.62 to 1.32% from 10 to 17 days of age (Expt 2).

[2] Technical Bulletin No. 680, Sigma Chemical Co., St. Louis, 1965.

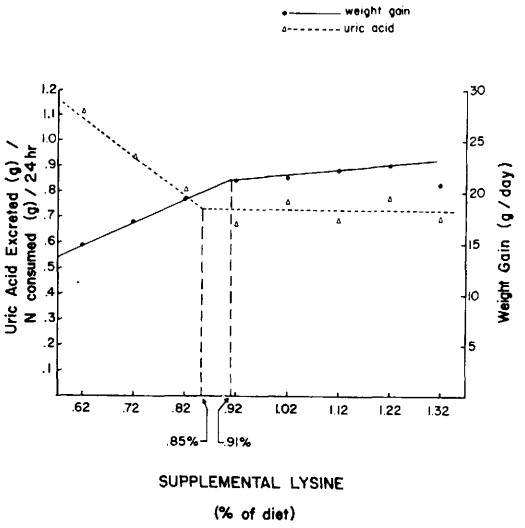

FIG. 3. Weight gain and uric acid excretion of chicks fed glucose–safflower meal diets with lysine contents ranging from 0.62 to 1.32% from 7 to 14 days of age (Expt 3).

data a value of 0.87% was observed (Fig. 1).

In Expt 2, lysine levels ranging from 0.62 to 1.32% were fed in a glucose–safflower meal diet to chicks from 10 to 17 days of age. Weight gain data indicated the lysine requirement to be 0.79% as compared with a value of 0.83% when uric acid excretion data were analyzed (Fig. 2).

Analysis of weight gain data from Expt 3 indicated a lysine requirement of 0.91% for chicks from 7 to 14 days of age (Fig. 3). Uric acid excretion data indicated a requirement of 0.85%. The reason for the higher values noted in Expt 3 as compared with Expt 2, particularly for weight gain, is not obvious. It is doubtful that the small difference in age of the birds would be that significant. The weight gain of chicks in Expt 3 fed the diet containing 1.32% lysine was decreased as compared with chicks fed diets containing the next lower levels of lysine. These results may suggest that this dietary level of lysine is becoming toxic and causing a slight growth depression. The weight gain of this group of chicks was not included in the least-squares analysis for the lysine requirement.

Analysis of plasma uric acid concentrations in Expt 3 showed a similar pattern as was noted for excreta uric acid (Table I). The highest concentration was noted with the lowest dietary lysine levels. Plasma uric acid concentrations decreased with higher levels of lysine and then plateaued at roughly the same dietary lysine level at which uric acid excretion plateaued.

Uric acid is formed from xanthine in birds by a reaction catalyzed by the NAD-dependent enzyme xanthine dehydrogenase. The activity of this enzyme in the livers of chicks in Expt 3 was determined (Table I). Lower xanthine dehydrogenase activity was noted in liver of chicks fed the lower 4 dietary levels of lysine as compared with those fed the 4 higher dietary levels. Whether these differences were statistically significant ($P < .05$) depended upon the basis of expressing enzyme activity. Higher levels of activity have previously been reported as a result of feeding high-protein diets to chicks and turkeys (3, 7). The changes in opposing directions of plasma uric acid and liver xanthine dehydrogenase activity do not agree with results of Scholz and Featherston (8) who noted similar changes in these two parameters in chicks fed 25 and 75% isolated soybean protein diets. The changes in enzyme activity noted

TABLE I. Plasma Uric Acid and Liver Xanthine Dehydrogenase Activity (Expt 3).

Dietary lysine (%)	Plasma uric acid[a] (mg/100 ml)	Xanthine dehydrogenase activity[b]	
		μmoles/100 g body wt/g nitrogen consumed	μmoles/g protein
0.62	25.22[d]	0.80[c]	0.016[d]
0.72	18.82[c,d]	0.88[c]	0.019[c,d]
0.82	18.78[c,d]	0.80[c]	0.018[c,d]
0.92	14.88[c]	0.86[c]	0.018[c,d]
1.02	14.07[c]	1.08[c]	0.022[c]
1.12	12.35[c]	1.13[c]	0.020[c,d]
1.22	13.93[c]	1.14[c]	0.022[c]
1.32	13.23[c]	1.09[c]	0.022[c]

[a] Values represent means of 6 observations each. Means in a column with the same lettered superscript are not statistically different ($P > 0.05$).

[b] Values represent enzyme activity catalyzing the reduction of 1 μmole of NAD/min at 25°.

in this study are small in comparison to those noted in previous studies, however, and adequate xanthine dehydrogenase activity may have been present to catabolize the relatively small amounts of nitrogen in these chicks as compared with chicks subjected to a three-fold increase in dietary nitrogen as in previous studies (8).

These studies indicate that uric acid excretion may serve as an accurate measure of the amino acid requirement of the chick. This approach may also be of value as an indicator of protein quality in birds where separation of urinary and fecal nitrogen for biological value determinations are extremely difficult.

Summary. Studies were conducted with chicks fed crystalline L-amino acid and glucose–safflower meal diets to compare uric acid excretion with weight gain as indicators of the lysine requirement of chicks. Uric acid excretion decreased and weight gain increased as dietary lysine was increased to the requirement level, at which point both parameters plateaued with only slight changes occurring as the dietary lysine levels were increased beyond that point. Least-squares analysis of the uric acid excretion and weight gain data indicated good agreement between the requirement values obtained from the two parameters. Plasma uric acid concentrations followed a similar pattern as was noted with excreta uric acid. Xanthine dehydrogenase activity, on the other hand, was somewhat higher in chicks fed the higher levels of lysine in a glucose–safflower meal diet. The results of these studies suggest that uric acid excretion may serve as an accurate indicator of the amino acid requirement of the chick.

1. Kiriyama, S., and Iwao, H., Agr. Biol. Chem. 33, 1483 (1969).

2. Brown, J. A., and Cline, T. R., J. Ani. Sci. 35, 211 (1972).

3. Scholz, R. W., and Featherston, W. R., J. Nutr. 95, 271 (1968).

4. Velu, J. G., Baker, D. H., and Scott, H. M., J. Nutr. 101, 1249 (1971).

5. Featherston, W. R., and Scholz, R. W., J. Nutr. 95, 393 (1968).

6. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics," McGraw-Hill Book Co., New York (1960).

7. Westerfeld, W. W., Richert, D. A., and Hermans, A. C., J. Nutr. 76, 475 (1962).

8. Scholz, R. W., and Featherston, W. R., J. Nutr. 98, 193 (1969).

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