Influence of Copper Supplementation on the Relationship between Dietary Methionine and Free Plasma Methionine

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ABSTRACT One-day-old broiler chicks were adapted to a basal, isolated soyprotein-cornstarch diet containing 20% protein, 0.59% methionine and 3,300 kcal metabolizable energy (ME)/kg. They were then fed experimental diets consisting of three levels (0, 500, 1,000 ppm) of copper added to each of the basal diet plus four levels (0, 0.4, 0.8, or 1.5%) of L-methionine for 1 or 4 weeks. Growth was retarded after 1 week by all levels of supplementary methionine, and by 500 or 1,000 ppm excess copper. The plasma concentrations of free methionine, serine, α-aminobutyric acid and cystathionine were increased by excess dietary methionine. Excess copper prevented the increase in plasma methionine. After 4 weeks, the plasma methionine concentration and rate of growth of chicks fed 0.4% excess L-methionine did not differ significantly from basal values. The growth retardation caused by 500 ppm excess copper was alleviated by 0.4% supplemental methionine, and the elevations in plasma methionine and liver and spleen iron concentrations observed in chicks fed 1.5% excess methionine were reduced by 1,000 ppm excess copper. The patterns of the relationship between dietary methionine and liver or spleen iron, in the presence or absence of supplementary copper, were similar to those between dietary and free plasma methionine. J. Nutr. 111: 1621–1629, 1981.

INDEXING KEY WORDS methionine · copper · toxicity

In an earlier study (1), a dietary supplement of methionine was shown to depress the levels of copper in the livers and plasmas of chicks fed various dietary concentrations of copper. Also, the hepatic and splenic hemosiderosis caused by 1.5% excess DL-methionine was apparently alleviated by 1,000 ppm supplementary copper. It is possible that the hemosiderosis was alleviated because copper reduced the concentration of methionine in the body. The present trial was conducted to assess that possibility, and to determine the influence of copper on the plasma concentration and metabolism of methionine.

MATERIALS AND METHODS
Chicks were reared as described earlier (2). However, we modified the basal diet by substituting L- for DL-methionine, doubling the iron content and including less vitamin E and choline chloride (table I). In the 2 × 3 × 4 factorial experiment, three levels (0, 500 and 1,000 ppm) of copper as CuSO₄·5H₂O were added to each of four levels (0, 0.4, 0.8 and 1.5%) of supplementary L-methionine and fed to broiler chicks for 1 or 4 weeks. The 144 chicks used were randomly assigned, at day-old, to 12 groups each of which consisted of two sets of six chicks. All were fed the basal diet for 12 days after which they were given the experimental

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TABLE 1
Composition of the basal diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>600</td>
</tr>
<tr>
<td>Isolated soy protein</td>
<td>230</td>
</tr>
<tr>
<td>Solka Floc (cellulose)</td>
<td>39</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>35</td>
</tr>
<tr>
<td>CaHPO₄·2H₂O</td>
<td>45</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>15</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>22.5</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>5.0</td>
</tr>
<tr>
<td>Choline chloride (50%)</td>
<td>4.5</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>4.0</td>
</tr>
</tbody>
</table>

1 Contains 20% protein (%N x 6.25), 3,300 kcal ME/kg (calculated and a total of 0.5% methionine.
2 Ralston Purina Co., St. Louis, MO. 3 Supplied the following in g/kg diet: NaCl, 5.0; KCl, 4.0; MgCO₃, 2.6; MnSO₄·H₂O, 0.2; CuSO₄, 5H₂O, 0.02; FeSO₄·7H₂O, 0.4; ZnO, 0.05; (CH₃CO)₂Co·4H₂O, 0.01; Na₂MoO₄·2H₂O, 0.01; KIO₄, 0.002; NaSeO₄·5H₂O, 0.0007.
4 Supplied the following in wt. or mg/kg diet: vitamin A, 5,000 IU; cholecalciferol, 1,000 IU; vitamin E, 50 IU; vitamin K, 2.7; thiamin hydrochloride, 9.0; riboflavin, 18.0; calcium pantothenate, 50; niacin, 135; pyridoxine mono-hydrochloride, 15; folacin, 6.0; biotin, 0.46; and vitamin B₁₂, 0.045.

RESULTS AND DISCUSSION

The results of the experiment are summarized in tables 2 and 3 and figures 1–6. The mean body weight gains of chicks were affected significantly (P < 0.01) by the length of time the chicks were fed the experimental diets and by the levels of dietary methionine or copper. The three factors interacted significantly in their effects. As shown in table 2, the amount of body weight gained in 1 week by chicks fed the basal diet was significantly greater than that of chicks fed the same diet supplemented with either 500 or 1,000 ppm copper. Chicks fed 500 ppm excess copper in turn gained more weight than those fed 1,000 ppm excess copper. The mean 1-week body weight gain of chicks fed the basal diet was also significantly greater than that of each of the groups of chicks fed the same diet supplemented with 0.4, 0.8 or 1.5% L-methionine. Chicks fed 0.4 or 0.8% excess L-methionine, gained more body weight than those fed 1.5% excess L-
methionine, but did not differ significantly among themselves.

After 4 weeks, no significant differences were observed among the body weight gains of chicks fed either the basal diet, the basal diet plus 0.4% L-methionine or the basal diet supplemented with both 0.4% L-methionine and 500 ppm copper. This observation is compatible with reports that growth depression caused by feeding 500 ppm excess copper to chicks (7, 8) or poult (9) for four or three weeks, respectively, was alleviated by supplementing the diet with 0.4% methionine. It is also compatible with the observation by Daniel and Waisman (10) that rats which adapted to excess dietary methionine gained body weight at near normal rates.

The concentration of iron in livers and spleens of chicks was affected significantly (P < 0.05) by the level of dietary methionine or copper. As shown in figure 1, the level of iron in the liver or spleen did not change with increases in the level of dietary methionine up to 0.8%, but increased significantly to about twice the basal value when 1.5% excess L-methionine was fed for 4 weeks. Harter and Baker (11) reported linear increases in chicken spleen iron concentrations only when the chicks ate diets with more than 1.0% excess L-methionine. The present results are compatible with this observation and also show that liver iron followed a similar trend. The concentration of iron in livers or spleens of chicks fed 500 or 1,000 ppm excess copper appeared lower, at every level of dietary methionine, than that of chicks fed the basal diet. It seemed that 1,000 ppm supplemental copper was more effective than 500 ppm in depressing iron levels, and it significantly alleviated the iron accumulation caused by 1.5% excess L-methionine.

An apparently inverse relationship between dietary copper and liver or spleen iron concentrations was also observed in an earlier study (1). However, the apparent increase in liver iron caused by 1,000 ppm excess dietary copper in that study was not observed in the present study. The reason for this discrepancy is not clear. A probable explanation may be differences in age or length of the period of exposure of birds to the copper-supplemented basal diet. In the previous study, the chicks were 6 days old before they were exposed to and fed the toxic diet for 1 week. The chicks in the present study were 12 days old before they were fed the toxic copper diet for 4 weeks.
ionine was affected significantly by the level of dietary methionine. As the level of dietary methionine increased, the concentration of free methionine in plasmas of chicks fed for 1 week rose and was significantly greater than the basal value when 0.8 or 1.5% excess L-methionine was fed. The pattern of this relationship between dietary and plasma methionine (fig. 2A) is akin to that reported by Harter and Baker (11) for 1-week-old chicks fed crystalline L-amino acid diets for 8 days. The concentration of plasma methionine in chicks fed 0.4% excess L-methionine for 4 weeks did not differ from the basal value. The increase caused by 0.8% excess dietary L-methionine was two-thirds of that of chicks fed the same diet for 1 week and did not differ significantly from the basal value. However, the level of plasma methionine in chicks fed 1.5% excess methionine was approximately twice that of chicks consuming the same diet for only 1 week, and was significantly more than that of chicks fed the basal diet (fig. 2).

These observations indicate that chicks consuming 0.4 or 0.8% excess L-methionine are, by the 4th week, capable of reducing their initially elevated levels of plasma methionine. This is compatible with the observations that rats adapt to excess dietary methionine with time (12–13). The pattern of the relationship between dietary methionine and plasma methionine in chicks fed for 4 weeks was similar (fig. 1, solid curves) to that already described between dietary methionine, and liver and spleen iron. An accumulation of iron in spleens and livers is a consistent observation in animals fed toxic levels of methionine.

It has been suggested that methionine exerts its toxic effects either by: 1) accumulating in the body; 2) depleting hepatic adenosine triphosphate (ATP); 3) inducing a secondary threonine deficiency; or 4) causing an accumulation of homocysteine in plasma and tissues. The toxic effects of the amino acid have also been associated with the metabolism of its methyl group. These suggestions have been discussed at some length elsewhere (14, 15). The concurrent marked

The effects of dietary treatments on the concentrations of free amino acids in plasmas of chicks fed the different diets for 1 or 4 weeks, are presented in figures 1–6.

The concentration of free plasma meth-
increases in the concentration of iron in spleens or livers, and of free methionine in plasmas, of chicks fed excess methionine in the present study, tend to support the hypothesis that the toxic effects of methionine are due to its accumulation in the body. The observation that 1,000 ppm copper significantly reduced the concentrations of both plasma methionine and liver or spleen, iron, lends further support to this hypothesis.

Excess dietary methionine also induced significant increases in the concentrations of free plasma serine, α-aminobutyric acid and cystathionine after 1 week and of threonine, serine and cystathionine after 4 weeks. These results are compatible with the observation of Miller et al. (16) that the concentration of free cystathionine and α-aminobutyric acid in plasmas of broiler chicks increased significantly with elevations in the level of dietary methionine. Girard-Globa et al. (12) and Sanchez and Swendsen (17) also observed a significant increase in free α-aminobutyric acid in plasma of rats fed excess methionine. Some investigators have reported decreases in the levels of glycine (17–19), serine (18) and threonine (12) in plasmas of rats fed excess dietary methionine. Miller et al. (16) also observed a drop in the concentrations of threonine and serine in
were also significantly influenced by the level of dietary copper. The influence of supplementary copper on the relationship between dietary methionine and the concentration of free methionine in plasmas of chicks fed for 1 or 4 weeks, is shown in figure 2. After 1 week, both 500 and 1,000 ppm supplementary copper reduced the concentration of methionine in plasmas of chicks fed 0.8% excess L-methionine to levels that were no longer significantly different from the basal value. The concentration of plasma methionine in chicks fed 1.5% excess L-methionine was reduced to a level not different from the basal value, only by 1,000 ppm copper. After 4 weeks, a similar depressant effect of 500 or 1,000 ppm copper on plasma methionine was observed only in chicks fed 1.5% excess L-methionine.

In a previous study (1), a supplement of 1.5% DL-methionine was shown to

chicks whose diets were supplemented with methionine.

The results of the present study do not agree with these reports. The diets used by Miller et al. (16) contained a high level of glutamic acid which, by itself, depressed threonine or serine levels. This may explain the difference in results. The disparity with the rat studies may be due to species differences. However, the results of other studies on rats fed excess methionine indicated no significant changes in plasma concentrations of glycine (12), serine (12, 17) or threonine (17).

The concentrations of free plasma methionine, serine, glycine, α-aminoisobutyric acid, cysteine and cystathionine
depress the level of copper in plasmas and livers of chicks fed various dietary levels of copper. A supplement of 0.4% methionine has also been shown in the present study and by Jensen and Maurice (7, 8) and Christmas and Harms (9), to alleviate the toxic effects of 500 ppm copper on the growth of chicks or pouls. These observations indicate an inverse relationship between copper and methionine. Since the completion of our studies, the results of other experiments that lend further support to the existence of this inverse relationship have been published (20, 27). The probability that this relationship does not occur to any significant extent when the level of copper or methionine is within the normal range, is indicated by the observation in the present study that the concentrations of methionine in plasmas of chicks which were fed the basal diet (fig. 2A) or had adapted to excess dietary methionine (fig. 2B), were not affected by supplementary copper. Also, the depressant effect of 1.5% excess dietary methionine on the concentration of copper in plasmas of chicks fed diets containing normal levels of copper was shown not to be statistically significant in previous studies (1, 2).

Supplementary copper could decrease the level of plasma methionine by stimulating an increase in the catabolism of the amino acid. If this was actually the case, increases in the concentrations of amino acids involved in, or produced as a result of the catabolism of methionine should accompany the resultant decrease in methionine concentration. The effects of supplementary copper on these amino acids are compared in figures 3–6, with the effects of copper on free plasma methionine. Cysteine was the only catabolite that seemed to have been increased by supplementary copper. The concentration of this amino acid in plasmas of chicks fed 0.8% excess L-methionine for 4 weeks, was significantly increased by 1,000 ppm copper. The concentration of free plasma threonine, glycine, serine, α-aminobutyric acid or cystathionine either remained unchanged or appeared to decrease as the concentration of dietary copper increased, and the pattern of these changes were fairly close to that observed for methionine (fig. 2).

Excess dietary methionine could decrease plasma or liver copper levels directly by chelating copper or indirectly by inducing the production and/or release of corticosteroids. Henkin (22) observed decreased plasma copper and increased urinary copper excretion in humans or cats given glucocorticoids or adrenocorticotropic hormone. As shown in the present trial (table 3), and by Girard-Globa et al. (12) and Munro et al. (23) excess dietary methionine stimulates an increase in the quantity of plasma glucocorticoids. While it is possible to ascribe the depressed copper levels caused by excess dietary methionine to an increase in the concentration of plasma corticosterone, it is doubtful if this can explain the depressant effect of copper on plasma methionine.
TABLE 3

Effect of dietary copper on the concentration of corticosterone in plasmas1 of broiler chicks fed a soyprotein-cornstarch diet containing 0, 0.4, 0.8 or 1.5% L-methionine. The chicks were 12 days old and fed the diets for 1 week

<table>
<thead>
<tr>
<th>Dietary copper level ppm</th>
<th>Level of supplementary L-methionine</th>
<th>% diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>0</td>
<td>3.8abc</td>
<td>4.7abc</td>
</tr>
<tr>
<td>500</td>
<td>3.2abc</td>
<td>2.8bc</td>
</tr>
<tr>
<td>1,000</td>
<td>2.3c</td>
<td>6.1abc</td>
</tr>
</tbody>
</table>

1 Since plasma samples from the six chicks in each group were pooled for corticosterone analysis, the “interaction” mean square was used instead of the “error” mean square in estimating F-ratios in two-way analysis of variance, or least significant ranges in Duncan’s new multiple-range test (see text).

**a** Data bearing different superscripts differ significantly (P < 0.05).

It has been shown (24) that amino acid-copper complexes are involved in the transportation of copper between various body compartments. Chelation, resulting in the formation of a methionine-copper complex that may be sequestered in other tissues or excreted, could explain the inverse relationship between copper and methionine.

It was shown in our previous study (1) that the antagonistic effects of 250 ppm copper and 1.5% DL-methionine on the concentrations of copper in livers, and of iron in livers and spleens, were not due to a reduction in feed intake. However, the results of that study and the present one did not eliminate the possibility that reductions in feed intake were responsible for the opposing effects of excess methionine and 500 or 1,000 ppm copper on these parameters.

LITERATURE CITED

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