Histopathological and Biochemical Effects
of Feeding Excess Dietary Methionine to Broiler Chicks

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SUMMARY 
Three experiments were conducted to determine the histological and biochemical effects of toxic levels of dietary DL-methionine on broiler chicks fed an isolated soy-protein/cornstarch-purified diet containing 20% protein, 0.59% methionine, and 3,304 kilocalories of metabolizable energy per kilogram. An appropriate level of supplementary DL-methionine to use in toxicity studies was found to be 1.5%. It significantly depressed (P < 0.01) gain in body weight, hematocrit, and hemoglobin concentration, increased (P < 0.05) the level of iron in liver and spleen, caused pancreatic damage, and induced neurological changes. Unlike the retarded growth and increased iron levels in spleen and liver, the fall in hematocrit and hemoglobin values was independent of the reduction in feed intake caused by excess dietary methionine.

INTRODUCTION 
The proteins of cereal grains and legumes constitute a major proportion of the total protein consumed worldwide. The nutritional value of these proteins, however, is limited by inadequate quantities of one or more of the essential amino acids. Lysine is the most limiting amino acid in cereal grains, and methionine is limiting in legumes (24). Fortifying the poorer-quality proteins with their limiting amino acids has been proposed as an effective, rapid, and economical way of upgrading the quality of such pro-

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...protein. This procedure is practiced on a large scale in Japan and to a lesser extent in some other southeast Asian countries, where flour, grains, bread, and noodles are fortified with L-lysine (24). It is also common to supplement poultry feeds with DL-methionine (20).

Excessive amounts of individual amino acids may be toxic (24). In 1942, Earle and co-workers (6) observed a severe retardation of growth and increased mortality in rats fed diets containing 6.4–12.8% DL-methionine. There have been several other reports since then on the toxicity of methionine for the rat (1,3,4,8,10,15, 16,17,18,19,22,27,29), chicken (9,11,14), turkey (9), guinea pig (10), rabbit (12), and human (25). In addition to retarded growth, other signs of toxicity observed were anorexia (1,14,29), depressed efficiency of feed conversion (3,8,11,14), reduced retention of nitrogen (25), decreased hepatic ATP (10,14), hypoglycemia (10), increased plasma (8,11,22) and hepatic (4,22) methionine, anemia (17,18), hepatic hemosiderosis (16), splenic hemosiderosis (1,16,27,29), and pancreatic acinar damage (16,27). Methionine is regarded as the most toxic of all essential amino acids: a diet containing two to three times the normal requirements of methionine affects animals adversely. The exact mechanism by which methionine exerts its toxic effects has not been established, although several theories (1,3,4,8,10,14,21,29) have been advanced to explain it.

Several observations suggest an interrelationship between excess dietary methionine and the concentration of copper in the body. Rats fed about 2.5% dietary L-methionine for 11 days had increased corticosterone levels in their plasma and adrenal glands (19). Humans and cats had raised plasma cortisol and urinary copper levels but depressed plasma copper levels when injected intravenously with cortisol (28). Toxic levels of dietary methionine (16,18) and a deficiency of copper (28) decreased the survival rate of red blood cells and induced iron to accumulate as hemosiderin in liver and spleen. Copper is a component of ceruloplasmin, the enzyme believed to be required for mobilizing iron from storage organs (28). Amino acids, including methionine, transport copper between various body compartments (23).

The present experiments with chicks establish a dietary level of supplementary methionine that decreases gain in body weight by about 50% without causing mortality. The effects of this level of excess dietary methionine on the histology, and the copper, iron, hemoglobin, or hematocrit status of the chicks were assessed.
MATERIALS AND METHODS

Three experiments were conducted using day-old broiler chicks (Foster Poultry Farms, Modesto, California) reared in electrically heated, thermostatically regulated Petersime brooder units (Petersime Incubator Company, Gettysburg, Ohio) housed in a room maintained at about 25.8°C. The room was uniformly illuminated with artificial lights for 14 hr daily. The chicks were provided with fresh distilled water daily and fed an isolated soy-protein/cornstarch-based purified diet in stainless-steel containers. The basal diet, the composition of which has been described (9), contained an equivalent of 20% protein (% N × 6.25), 3,304 kcal of metabolizable energy per kilogram, and was supplemented with 0.4% DL-methionine. More methionine added to the diet replaced an equivalent amount of cornstarch.

The first experiment was designed to determine the amount of supplemental methionine that would depress body weight gain by about 50% without causing mortality. Three groups of chicks were reared for 12 days on either the basal diet alone or on the basal

![Graph](image)

Fig. 1. Mean body weight gains of three groups of one-day-old broiler chicks fed a soy-protein/cornstarch diet, alone or with 0.8% or 1.5% DL-methionine, for 12 days. Eight chicks per group, each group duplicated.
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Table 1. Mean hematocrit and hemoglobin, and the iron and copper concentrations of organs of one-week-old broiler chicksa fed a soy-protein/cornstarch diet, alone or with 1.5% DL-methionine, for four weeks.

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Basal diet</th>
<th>Basal diet + 1.5% DL-Methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>30.5 ± 2.6(^{a,b})</td>
<td>26.9 ± 1.0(^{b})</td>
</tr>
<tr>
<td>Hemoglobin (g/100 ml)</td>
<td>10.2 ± 0.7(^{a})</td>
<td>8.7 ± 0.4(^{b})</td>
</tr>
<tr>
<td>Plasma copper (µg/100 ml)</td>
<td>19.8 ± 7.8(^{a})</td>
<td>14.0 ± 7.8(^{a})</td>
</tr>
<tr>
<td>Brain copper (µg/g)</td>
<td>2.3 ± 0.2(^{a})</td>
<td>2.9 ± 0.2(^{a})</td>
</tr>
<tr>
<td>Liver copper (µg/g)</td>
<td>3.7 ± 0.4(^{a})</td>
<td>4.2 ± 0.4(^{a})</td>
</tr>
<tr>
<td>Liver iron (µg/g)</td>
<td>69.5 ± 5.4(^{a})</td>
<td>135.3 ± 32.0(^{b})</td>
</tr>
<tr>
<td>Spleen iron (µg/g)</td>
<td>58.7 ± 11.0(^{a})</td>
<td>115.8 ± 54.1(^{a})</td>
</tr>
<tr>
<td>Neurological signs(^{c})</td>
<td>None</td>
<td>Present</td>
</tr>
</tbody>
</table>

\(^{a}\)Six chicks per group. Each group triplicated. Values expressed as mean ± standard deviation of mean.

\(^{b}\)Figures in the same row bearing different superscripts differ significantly (P < 0.01 or P < 0.05*).

\(^{c}\)See text for description of signs.

diet supplemented with 0.8% or 1.5% DL-methionine. Each group was duplicated and contained 8 chicks, which were individually weighed to the nearest gram at the beginning of the experiment, at three-day intervals, and at the end of the experiment.

The second experiment was designed to assess the pathological effect of an excess of dietary methionine and its effect on copper in the chicken. Thirty-six chicks were fed a commercial chicken starter diet for 7 days. On day 8, they were wing-banded, weighed to the nearest gram, and divided randomly into two triplicated groups of 6 birds each. One group was fed the basal purified diet. The other group was fed the basal diet supplemented with 1.5% DL-methionine. The chicks were reared for four weeks and weighed individually every other day during the first week and at about three-day intervals in subsequent weeks. After their final body weights had been recorded, the chicks were killed and blood was collected by 20-gauge 1-inch needles from the heart in glass bottles containing heparin-fluoride as an anticoagulant. Hematocrit was determined using an International micro-capillary centrifuge and reader (International Equipment Co., Boston, Massachusetts). Hemoglobin was determined by the cyanmethemoglobin method (7), and plasma was separated by centrifugation at 3,000 rpm for 10 min. in a Sorvall RC2-B automatic refrigerated centrifuge (Ivan Sorvall Inc., Norwalk, Connecticut 06856) kept at 5 C. Plasma copper was assayed as described (7). Organ samples from
the first triplicate of each group were collected and preserved in 10% neutral-buffered formalin. Two slides were prepared from each organ sample (7). One slide was stained with hematoxylin and eosin. The other was stained for iron as described (7). Liver, spleen, and brain samples from the second and third triplicates were collected in plastic bags kept in ice; they later were frozen. The iron and copper contents of pooled samples of these organs (three samples per pool) were determined by atomic absorption spectrophotometry as described (7).

The third experiment was designed to determine whether toxicity signs were due to methionine, or whether they were a result of reduced feed intake caused by an excess of methionine. Two groups of 10 chicks were fed the basal diet for the first week. A third group was fed a commercial chick-starter diet in error. The first group was continued on the basal diet for 8 more days, the

![Diagram](https://via.placeholder.com/150)

Fig. 2. Mean body weight gains of two groups of one-day-old broiler chicks fed a soy-protein/cornstarch diet, with (▲) or without (○) DL-methionine, for 4 weeks. Six chicks per group, each group triplicated.
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Second group was switched to the basal diet supplemented with 1.5% DL-methionine, and the third group was pair-fed to the second group. Body weight gains were determined, and blood, liver, and spleen samples were collected and analyzed as described in the second experiment, except that liver samples were not pooled.

Data were analyzed by the analysis of variance, Duncan's new multiple-range test, and correlation coefficient (26).

RESULTS

Expt. 1. The one-week body weight gains of chicks were depressed to 86% and 48% of normal by diets containing 0.8% and 1.5% excess DL-methionine, respectively (Fig. 1). The effect of 1.5% DL-methionine was statistically significant ($P < 0.01$).

Expt. 2. After 10 days on the diets, the chicks fed 1.5% excess DL-methionine became lethargic. They gazed constantly at the cage floor and were ataxic and hyperexcitable to various degrees. The birds affected most seriously went into convulsive seizures when touched, picked up and dropped, or distracted by sudden loud noises. These symptoms worsened with time and were still present by the time the chicks were killed. As shown in Fig. 2 and Table 1, the body weight gain and hematocrit and hemoglobin concentration of chicks fed 1.5% excess DL-methionine were depressed significantly ($P < 0.01$). Excess dietary methionine also induced changes in the concentration of copper and iron in various organs. The concentration of copper was decreased in plasma and increased in liver and brain, but these changes were not statistically significant. There was a significant ($P < 0.05$) increase in the concentration of iron in the livers of chicks fed 1.5% excess DL-methionine and an apparent increase in the level of iron in their spleens.

Except for darkened spleens and livers, the gross appearance of the organs of chicks fed 1.5% excess DL-methionine was normal. Light microscopy was used to examine sections of the esophagus, crop, proventriculus, gizzard, duodenum, jejunum, ileum, cecum, colon, bursa of Fabricius, trachea, lungs, heart, liver, gall bladder, spleen, pancreas, kidney, adrenal glands, and testes. There was distortion of parenchymal architecture and degeneration of acinar cells caused by loss of zymogen granules from the pancreas of chicks fed 1.5% excess DL-methionine (Fig. 3). The spleens of these chicks were observed to have hemosiderin deposits and hyperplasia of the reticuloendothelial cells (Fig. 4). Hemosiderin deposits were also present in one of the liver samples (Fig. 5). The
Fig. 3. Pancreas. (a,c): Chicks fed the basal diet. (b,d): Chicks fed 1.5% excess DL-methionine—disruption of acinar structure and degeneration due to loss of zymogen granules. H & E stain. a,b: ×210; c,d: ×480.
Fig. 4. Spleen. (e,g): Chicks fed the basal diet. (f,h): Chicks fed 1.5% excess DL-methionine—hemosiderosis and hyperplasia of reticuloendothelial cells. Gomori stain. e,f: ×85; g,h: ×210.
Table 2. Mean body weight gains, hematocrit, and hemoglobin, and the iron and copper concentrations of organs of one-week-old broiler chicks fed a soy-protein/cornstarch diet, alone or with 1.5% DL-methionine, for one week.

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Basal diet</th>
<th>Basal diet + 1.5% DL-methionine</th>
<th>Basal diet pair-fed to methionine group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g)</td>
<td>88.9 ± 32.9a b</td>
<td>35.4 ± 14.9b</td>
<td>27.8 ± 13.1b</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>25.5 ± 2.0a</td>
<td>21.0 ± 3.5b</td>
<td>27.1 ± 3.0a</td>
</tr>
<tr>
<td>Hemoglobin (g/100 ml)</td>
<td>8.5 ± 0.8a</td>
<td>6.7 ± 1.7b</td>
<td>10.6 ± 1.5ca</td>
</tr>
<tr>
<td>Plasma copper (µg/100 ml)</td>
<td>15.0 ± 2.9a</td>
<td>8.9 ± 4.7ab</td>
<td>4.1 ± 2.0b</td>
</tr>
<tr>
<td>Liver copper (µg/g)</td>
<td>4.0 ± 0.8a</td>
<td>4.9 ± 1.1a</td>
<td>6.7 ± 0.9g</td>
</tr>
<tr>
<td>Liver iron (µg/g)</td>
<td>81.2 ± 33.0a</td>
<td>241.4 ± 118.3b</td>
<td>144.0 ± 27.9b</td>
</tr>
<tr>
<td>Spleen iron (µg/g)</td>
<td>94.0 ± 33.8a</td>
<td>270.6 ± 79.9b</td>
<td>183.7 ± 34.8b</td>
</tr>
</tbody>
</table>

a Ten birds per group. All, except those in the “pair-fed” groups, were fed the basal diet from one day to one week of age. The pair-fed group was fed a commercial chicken starter diet in error. Values expressed as mean ± standard deviation.

Figures bearing different superscripts in each row differ significantly (P < 0.01 or P < 0.05a).

degree of hemosiderosis in spleens varied from heavy and extensive in one sample to mild and scattered in three others. Hepatic hemosiderosis was diffuse and confined mainly to the Kupffer cells. Apart from these and the atrophic changes observed in the mucosa of different sections of the gastrointestinal tract, all the organs examined appeared normal.

Expt. 3. Results (Table 2) confirm the findings of Expt. 2 as to the effects of 1.5% excess dietary DL-methionine on body weight gain, plasma copper, liver copper, and the concentration of iron in the spleen and liver. These effects may be attributable, totally or partly, to the reduction in feed intake caused by an excess of the amino acid because the direction, and in some cases the magnitude, of change in the value of the measured parameter is the same in the group of chicks fed the toxic diet and in those pair-fed to them. Conversely, the fall in hematocrit or hemoglobin level is independent of the feed-intake effect. There was a negative but non-significant correlation \((r = -0.4)\) between plasma copper and liver iron concentrations. The ranges of iron levels in the liver and

Fig. 5. Liver. (j): Chicks fed the basal diet. (k): Chicks fed 1.5% excess DL-methionine—hemosiderin granules few and limited to Kupffer cells (see arrows). Gomori stain. ×270.
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spleen respectively were 29.7 to 144.7 µg/g and 58.1 to 125.3 µg/g for chicks fed the basal diet, 114.3 to 488.6 µg/g and 214.1 to 327.1 µg/g for those eating the toxic diet, and 93.4 to 182.2 µg/g and 143.6 to 205.9 µg/g for the chicks pair-fed to the methionine group.

DISCUSSION

The histopathological effects of excess dietary methionine in rats have been studied extensively. The lesions described include damage to or degeneration of pancreatic acini (15,16,27), dilation of renal tubules (16), sinusoidal engorgement and hemosiderosis of spleen (1,16,29) and accumulation of hemosiderin, mostly in the Kupffer cells of an otherwise normal liver (16). Stekol and Szaran (27), however, have described degenerative and regenerative changes in the liver, and Earle et al. (6) and Benevenga et al. (1) observed no changes in the pancreas. It is not clear whether these differences in the reported effects of methionine on liver and pancreas can be attributed to differences in strain in rats, duration of experiment, level of dietary protein, or the level and form (DL or L) of methionine used. Kaufman et al. (15) and Klavins et al. (16) used male albino Sprague-Dawley rats fed 2.0-4.5% excess DL-methionine in an 18% casein diet for four weeks, and Benevenga and co-workers (1) fed a 10% casein diet containing 2.7% excess L-methionine to male Holtzman rats for up to 20 days. Stekol and Szaran (27), on the other hand, fed a 24% casein diet containing various levels (0.5%, 1.0%, 2.0%, and 4.0%) of excess D- or L-methionine to male or female Wistar-Carworth rats for one, three, four, or nine months.

The present study appears to be the first investigation of the histological effects of toxic levels of dietary methionine in chickens, and the lesions observed in the spleen, liver, and pancreas are similar to those reported for rats by most investigators. The observed decrease in hematocrit or hemoglobin concentration also agrees with reports in rats of Cohen et al. (3), Mengel and Klavins (18), and Klavins et al. (17), and with reports in chicks of Harter and Baker (11), but it is at variance with the remark by Van Pilsum and Berg (29) that the hemoglobin concentration of rats fed excess dietary methionine showed no marked divergence from normal. Zymogen granules are the precursors of pancreatic enzymes, and their reduction or loss may indicate either decreased synthesis resulting from damage to the pancreatic acini, or increased rate of conversion to pancreatic enzymes for use in the small intestine. Rapid incorporation of methionine into pancreatic acinar cells has been reported, and Kaufman et al. (15) have sug-
gusted that the damaging effects of excess methionine were due to the presence of unusually large quantities of methionine or its metabolite acting directly upon the acinar cells. Excess iron is stored as ferritin, or as hemosiderin when the quantity of excess iron is large. The presence of hemosiderin in the spleens or livers of chicks consuming a diet containing normal levels of iron indicates a derangement of iron metabolism. Mengel and Klavins (18) have shown a decrease in the survival time of erythrocytes of rats fed excess methionine. Harter and Baker (11) have demonstrated a direct relationship between increased dietary methionine, decreased hemoglobin, and increased splenic iron concentration. A marked increase in the quantity of iron determined by chemical methods was observed in the spleens of rats (3) and chicks (11) fed diets containing toxic levels of methionine. Our results are compatible with this observation and demonstrate that excess dietary methionine also induces a significant increase in the iron content of chick livers.

Phear et al. (21) noted that humans with cirrhosis of the liver became confused and mentally disoriented when given 14 to 18 times the normal daily adult requirement of methionine, and that schizophrenic patients exhibited behavior changes when given up to 36 times the daily adult requirement for seven days. These neurological changes may have been due to an accumulation of excess nitrogen as ammonia, because other nitrogenous compounds such as high-protein diets, ammonium salts, and urea induced similar changes. However, Phear et al. (21) observed that the neurological deterioration induced by excess methionine occurred without a significant change in blood ammonia.

The enzootic neonatal ataxia of lambs, a nervous disorder characterized by uncoordinated movement, has been associated with subnormal levels of copper in pastures and in the blood and tissues of both ewes and affected lambs (28). The low concentration of copper in the brain, liver, and heart leads to a deficiency of cytochrome oxidase in the organs and to an inhibition of phospholipid synthesis (28). Underwood (28) suggested that the ataxia is an expression of a myelin aplasia resulting from the deficiency of phospholipids, and reported neonatal ataxia and similar abnormalities in copper-deficient goats, pigs, newborn guinea pigs, and offspring of female rats fed a copper-deficient diet.

In the present study, chicks fed 1.5% excess DL-methionine for four weeks exhibited ataxia and other neurological changes. The concentration of copper in the plasma of these chicks was also
lower than in that of chicks fed the basal diet. Although this fall in plasma copper level was not significant statistically, one cannot totally ignore the possibility that the neurological changes observed are the result of a methionine-induced hypocupraemia. Moreover, methionine has been reported to lessen copper toxicity since the present study was completed (2,13). The relationship between methionine and copper needs further investigation.

The neurological effects of excess methionine may also be attributed to a deficiency of pyridoxine, which, in its coenzyme forms, is heavily involved in decarboxylation, transamination, and desulfhydration reactions of protein and amino acid metabolism. Pyridoxine is also needed for certain important chemical reactions that occur in the brain (5), and its deficiency causes behavioral disorder, hyperirritability, and abnormality of performance (20). The need to metabolize larger-than-normal quantities of methionine may increase the demand for the vitamin for this purpose and decrease its availability for use in the brain.

Because a marked increase in the concentration of free methionine in the plasma (8,11,21,22) and liver (4,22) appears to be a consistent finding in rats, chickens, and human liver-cirrhosis patients given large oral doses of methionine, methionine itself, and not a by-product of its metabolism as suggested by Phear et al. (21), may be responsible for the neurological changes.

The large variations in the intensity of neurological signs and in the quantity of histochemically or chemically determined iron present in the liver and spleen probably indicate differences in individual susceptibility of chicks to the toxic effects of methionine.

REFERENCES
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