Dietary Copper Influences Reproductive Efficiency of Queens

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EXPANDED ABSTRACT

Copper, an essential trace element for all animals, functions as a component of metalloenzymes that catalyze electron transport reactions involving molecular oxygen (Linder 1996). Cuproproteins have several enzymatic functions including copper transport, temporary storage and coagulation (Linder 1996).

Before the initiation of this project, there was only one published report concerning the copper requirements of cats (Doong et al. 1983). On the basis of that study and the requirement for rats, the National Research Council (NRC 1986) proposed a copper requirement of 5 mg Cu/kg diet for kittens for growth. However, while testing commercial diets in Association of American Feed Control Officials (AAFCO) protocols, we found that queens fed three different diets produced offspring with signs compatible with clinical copper deficiency in other species, including neonatal death, premature kittens, hypochromatricia and collagen abnormalities. All of these diets contained supplemental copper supplied as cupric oxide, a form demonstrated to be unavailable in other species (Baker et al. 1991, Cromwell et al. 1989). The objectives of this study were to determine the copper requirement of queens for gestation and the extent to which cupric oxide is an available form of copper for queens for reproduction.

Materials and methods. The experimental protocol adhered to the NRC guidelines (NRC 1985) and was approved by the Animal Use and Care Administrative Advisory Committee of the University of California at Davis.

Animals and their management. Thirty-eight 2- to 3-yr-old specific-pathogen–free domestic short-hair queens from the Feline Nutrition and Pet Care Center of the University of California at Davis were used. Queens were group housed in large wire cages (2.5 × 2.5 × 2.5 m) in rooms with controlled temperature (21 ± 2°C) and a light:dark cycle of 14 h:10 h. Queens were transferred to queening cages (76 × 76 × 76 cm) before giving birth and remained there until the kittens were weaned. Queens had free access to the experimental diets and water.

Diets. Experimental diets were prepared by adding varied amounts of copper as copper sulfate (CuSO₄ · 5H₂O) or cupric oxide (CuO), (both obtained from Fisher Scientific, Fair Lawn, NJ) to a casein/lactalbumin-based diet composed of the following constant ingredients (g/kg diet): casein (New Zealand Milk Products, Petaluma, CA), 220; lactalbumin (see casein), 220; rendered animal tallow (Florin Tallow, Dixon, CA), 270; sucrose, 75.5; starch (cornstarch, Melojoel, Bridgewater, NJ), 144; taurine (Taisho Pharmaceutical, Torrance, CA), 1.5; choline chloride (International Mineral and Chemical, Terre Haute, IN), 3; vitamin mixture (Williams et al. 1987), 10; L-methionine (Ajinomoto USA, Raleigh, NC), 3; L-arginine (Ajinomoto USA), 3; mineral mixture, 50 (containing CaHPO₄, 28.1; MgSO₄, 2.25; NaCl, 5.11; KCl, 13.85; MnSO₄ · H₂O, 0.153; ZnSO₄ · 7H₂O, 0.178; FeSO₄ · 7H₂O, 0.318; pentacalcium orthoperiodate, 0.0033; SnSO₄, 0.038; Na₂SeO₃, 0.0012; (NH₄)₆Mo₇O₂₄ · 4H₂O, 0.0016; CrCl₃ · 6H₂O, 0.00104; NiCl₂ · 6H₂O, 0.012; NaF, 0.0056; NH₄VO₃ · 4H₂O, 0.00008). The purified diet exceeded NRC recommended levels of all ingredients other than copper and has been demonstrated to support adequate growth and reproduction in cats at the Feline Nutrition and Pet Care Center. Copper concentrations in the experimental diets were confirmed by using an atomic absorption spectrophotometer (model 3030B, Perkin-Elmer, Clay Adams, NJ). Diets were stored at 4°C between preparation and feeding.

Design. A depletion-repletion study design was used. Queens were trained to eat a purified diet containing copper. When weight maintenance was satisfactory, they were fed a copper-depletion purified diet (0.8 mg Cu/kg diet) for 4 mo after which they were randomly allocated into one of four dietary treatment groups. Dietary copper additions to the purified diet were 3, 6 or 10 mg Cu/kg diet as copper sulfate, or 10 mg Cu/kg diet as cupric oxide. Plasma samples were collected every other week throughout the study. Five of the 38 queens underwent exploratory laparotomies for liver biopsies before and after the depletion and repletion periods. Plasma and liver samples were analyzed for copper concentration by using atomic absorption spectrophotometry (model 3030B, Perkin-Elmer). After the introduction of a tom into each
dietary treatment group at wk 4, queens underwent ultrasound screening weekly to ascertain pregnancy status, and throughout gestation to document resorptions or abortions. At parturition, kittens were weighed and given a physical examination for birth defects. Kittens born dead or that died after birth were necropsied, and a sample of liver was analyzed for copper. All queens were exposed to the toms to permit two pregnancies.

Statistical analysis. Statistical analyses were performed using PC-SAS (version 6.04, SAS Institute, Cary, NC). One-way ANOVA was used to test for differences in means, and a Bonferroni post-hoc test was used for comparisons. Differences were considered significant at \( P \leq 0.05 \). Least-squares regression analysis of dietary copper concentration, supplied as copper sulfate, and the time until the onset of gestation was computed.

Results. Plasma copper concentration did not reflect the copper intake of the queens. The mean plasma copper concentrations of all 35 queens before depletion and after 4 mo of consuming the low copper diet (0.8 mg Cu/kg diet) were 11.2 and 11.3 \( \mu \text{mol Cu/L} \), respectively. After the queens had received the dietary treatments for 1 y, there was no difference in plasma copper concentrations (Table 1).

The mean liver copper concentration in the five queens that underwent biopsies before the initiation of the low copper diet was 0.91 \( \pm 0.09 \) \( \mu \text{mol Cu/g tissue} \), and the mean decreased to 0.30 \( \pm 0.06 \) \( \mu \text{mol Cu/g tissue} \) after this diet was consumed for 4 mo (Table 1). A third biopsy, obtained from four of the queens before they were removed from the study, indicated that liver copper was responsive to changes in dietary copper intake, increasing with increased copper sulfate concentrations. The queen consuming the cupric oxide diet (10 mg Cu/kg diet) had an overall decline in liver copper concentration from 1.26 \( \mu \text{mol Cu/g tissue} \) before the study, to 0.42 \( \mu \text{mol Cu/g} \) after copper depletion, to 0.13 \( \mu \text{mol Cu/g} \) at the end of the study. There were no differences in liver copper concentrations in dead kittens among dietary groups, even when compared with kittens that died born to queens consuming the standard colony dry diet.

Reproductive rates were significantly affected by dietary treatments (Table 2). Only 47% of the queens consuming the diet that contained 10 mg Cu/kg as cupric oxide became pregnant, whereas the queens in groups consuming diets with copper sulfate had pregnancy rates >82%. The amount and form of copper had a significant effect (\( P \leq 0.04 \)) on the time for conception (defined as days from the introduction of a proven tom until conception). Queens consuming the cupric oxide diet had the longest interval between the introduction of a tom and the onset of gestation, 47 d. This interval was significantly greater than that for the queens consuming the 6 and 10 mg Cu/kg diets. There was a significant, inverse linear relationship between the dietary concentration of copper supplied as copper sulfate and the mean time required for those queens to become pregnant (\( y = 43.83 - 2.87x \), where \( x \) = mg/kg copper and \( y \) = days; \( R^2 = 0.97 \)). There was no significant difference between dietary treatment groups in the number of resorptions or abortions, number of kittens born per litter, birth defects, kitten mortality or birth weight.

Discussion. Among the variables measured, liver copper concentration was most sensitive to dietary intake and seemed the best reflection of copper stores in the queen but not in the kitten. This finding is similar to reports in other species (Klevay and Medeiros 1996) and supports the conclusions of the previous research by Doong et al. (1983). After 4 mo of consuming the copper-depletion diet, liver copper concentrations declined without any other accompanying signs of copper deficiency.

### Table 1

<table>
<thead>
<tr>
<th>Time point in study</th>
<th>Plasma copper1</th>
<th>Liver copper2</th>
<th>Plasma copper2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu \text{mol/L} )</td>
<td>( \mu \text{mol/g} )</td>
<td>( \mu \text{mol/L} )</td>
</tr>
<tr>
<td>Predepletion</td>
<td>11.2 ± 1.9</td>
<td>0.91 ± 0.09</td>
<td>9.9 ± 0.63</td>
</tr>
<tr>
<td>End of depletion</td>
<td>11.3 ± 1.9</td>
<td>0.30 ± 0.06</td>
<td>11.2 ± 1.3</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Dietary Cu concentration</th>
<th>Number of queens</th>
<th>Pregnancy rate, %</th>
<th>Mean days until onset of pregnancy2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuSO4</td>
<td>3 mg/kg</td>
<td>83</td>
<td>36 ± 26\textsuperscript{32}</td>
</tr>
<tr>
<td>CuSO4</td>
<td>6 mg/kg</td>
<td>82</td>
<td>25 ± 18\textsuperscript{32}</td>
</tr>
<tr>
<td>CuSO4</td>
<td>10 mg/kg</td>
<td>87</td>
<td>16 ± 12\textsuperscript{32}</td>
</tr>
<tr>
<td>CuO</td>
<td>10 mg/kg</td>
<td>47</td>
<td>47 ± 47</td>
</tr>
</tbody>
</table>

1 Data in this table are based on exposure of each queen to a tom to permit two pregnancies.
2 Significant effect of treatment, based on ANOVA, \( P \leq 0.04 \).
3 Three mg Cu/kg diet as copper sulfate compared with 10 mg Cu/kg diet as copper sulfate, \( P \leq 0.05 \).
4 Six mg Cu/kg diet as copper sulfate compared with 10 mg Cu/kg diet as cupric oxide, \( P \leq 0.04 \).
5 Ten mg Cu/kg diet as copper sulfate compared with 10 mg Cu/kg diet as cupric oxide, \( P \leq 0.01 \).
were abnormal. The mean hematocrit for the five queens that underwent biopsies was 33%. A third liver biopsy after dietary treatment for 1 year demonstrated an increase in liver copper concentration when dietary copper was supplied as copper sulfate. The lack of response in the queen consuming the cupric oxide diet, demonstrated by a continued decline in liver copper concentration, indicates that cupric oxide is a poorly available form of copper for queens.

Plasma copper concentration was not a good indicator of dietary copper intake in this study. This conclusion is similar to findings in other species (Milne 1994). The decline in liver copper concentrations in the five queens that underwent exploratory laparatomies was not accompanied by any parallel changes in plasma copper concentrations. Plasma copper concentrations may be maintained at the expense of other storage sites and may change only with excessive supplementation or depletion.

The form and amount of dietary copper not only influenced liver copper stores, but also reproductive efficiency in the queen. Queens consuming diets with copper in the form of copper sulfate had a higher pregnancy rate than queens consuming copper in the form of cupric oxide. It also took significantly less time for these queens to become pregnant when the diet contained copper sulfate supplied at a concentration of 6 mg and 10 mg of available Cu/kg diet. This finding suggests that the copper requirement for the queen for gestation should be at least 6 mg Cu/kg diet. The optimal amount of copper may be even higher based on the strong correlation between the amount of copper as sulfate, in the range of 3–10 mg Cu/kg in the diet, and the mean time it took queens to become pregnant ($R^2 = 0.97$).

The lack of clinical signs characteristic of copper deficiency (angular limb abnormalities and hair coat changes) in kittens from queens receiving the low levels of dietary copper was not anticipated. The lower pregnancy rate in the queens consuming the cupric oxide diet suggests that this poorly available form of copper inhibited gestation and did not allow the expression of congenital defects. We have observed clinical signs of copper deficiency in kittens when queens were abruptly changed from a diet adequate for gestation to one with copper supplied as cupric oxide. With the exception of one queen, all of the other pregnant queens in the cupric oxide dietary treatment group consumed their kittens immediately after parturition. It is unknown how many of these kittens were abnormal.

There were no significant differences in the liver copper concentrations among the kittens born dead or that died after birth. A plausible explanation may be that regardless of the queen’s stores, copper transport to the fetus is a priority. Unlike most other species (Luza and Speisky 1996), liver copper concentrations in the kittens were lower than those in the queens.

The results of this preliminary study indicate that liver, but not plasma copper concentrations are responsive to dietary intake of copper in cats. The current NRC recommendation of 5 mg Cu/kg diet is probably marginal for the queen for optimal gestation, especially if the copper is from a source with an availability lower than that of copper sulfate. Cupric oxide does not supply a biologically available form of copper for the cat and should not be used in any foods formulated for this species.

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**LITERATURE CITED**


