EFFECTS OF COPPER AND MOLYBDENUM SUPPLEMENTS ON THE COPPER AND SELENIUM STATUS OF PREGNANT EWES AND LAMBS

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ABSTRACT

The aim of this work was to investigate whether Cu, alone or in combination with Mo, reduces the Se status of sheep. Thirty-six Hampshire ewes were separated prior to mating into four treatment groups of nine sheep each. The four groups consisted of 1) basal (alfalfa hay or legume-grass low-moisture silage, corn, oats), 2) basal plus 10 mg Cu/kg diet, 3) basal plus 10 mg Mo/kg and 4) basal plus Cu and Mo (each 10 mg/kg). Copper and Mo were added to both ewe and lamb diets. Treatments continued until lambs were weaned at 10 wk postpartum. The basal ewe diet contained, in mg/kg, 5.6 Cu, .05 Se and 1.0 Mo. The basal lamb diet contained, in mg/kg, 6.1 Cu, .07 Se and <1.0 Mo. Sulfur concentrations in the ewe and lamb diets were .24% and .20%, respectively. Copper supplements increased, and Mo supplements decreased, the concentration of Cu in the livers of ewes and lambs (P < .05). Despite this, there were no effects of treatment on Se concentration or glutathione peroxidase activity in blood or tissues. Selenium levels in plasma of all ewes declined throughout pregnancy and lactation, indicating that .05 mg/kg Se was insufficient for the maintenance of Se status during pregnancy in Hampshire ewes. Copper and Se levels (mg/kg fresh weight) in the liver of lambs at weaning for Treatments 1 through 4 were 48 and .13, 158 and .10, 11 and .11, and 136 and .13, respectively. The results show that Cu or Mo supplements at 10 mg/kg to practical-type diets of ewes and lambs had no effect on Se status.

(Key Words: Sheep, Copper, Molybdenum, Selenium, Glutathione Peroxidase.)


Introduction

A dietary concentration of .1 mg Se/kg has been accepted by the NRC (1985) as a safe and adequate level for the prevention of white muscle disease (WMD) in sheep. There is, however, nearly a 10-fold difference in reports of what constitutes an adequate level. For example, workers in New Zealand found that lambs grew normally with no signs of WMD when pastures contained .03 to .04 mg Se/kg (Hartley and Grant, 1961). Oh et al. (1976) reported that .12 mg/kg diet gave optimal results in terms of both the maximal activity in tissues of the seleno-enzyme, glutathione peroxidase (GSHpx), and the minimal activity of plasma creatine kinase, an indicator of muscle damage in WMD. Oldfield et al. (1963) stated that .02 mg Se/kg diet was extremely dystrophic and suggested that .06 mg/kg was the minimal requirement. This agrees with the observations of Australian workers who showed that outbreaks of WMB in lambs were confined to areas where pastures contained less...
## TABLE 1. MINERAL COMPOSITION OF FEEDS AND SUPPLEMENTS FED TO EWES

<table>
<thead>
<tr>
<th>Item</th>
<th>Mo, mg/kg</th>
<th>Cu, mg/kg</th>
<th>Se, mg/kg</th>
<th>S, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa hay</td>
<td>&lt;1.0</td>
<td>9.4</td>
<td>.10</td>
<td>.27</td>
</tr>
<tr>
<td>Legume-grass silage</td>
<td>1.0</td>
<td>5.6</td>
<td>.05</td>
<td>.24</td>
</tr>
<tr>
<td>Grain (corn:oats, 1:3)</td>
<td>&lt;1.0</td>
<td>3.9</td>
<td>.04</td>
<td>.18</td>
</tr>
<tr>
<td>Creep$^a$ plus salt/mineral mix$^b$</td>
<td>&lt;1.0</td>
<td>6.1</td>
<td>.07</td>
<td>.20</td>
</tr>
</tbody>
</table>

$^a$Creep feed consisted of 20% ground alfalfa hay, 58.5% ground corn, 20% oats, 1% dicalcium phosphate, 5% salt mixture and antibiotic (.01% Aureo S-100). The composition of the salt mixture is described below.

$^b$The minerals were fed to ewes in a loose mix. For lambs, it was mixed with the creep feed. It contained 98.98% NaCl, 4% zinc carbonate, 4% ferric ammonium citrate, 2% manganous chloride, 0.1% potassium iodide and 0.1% cobaltous carbonate.

than .05 mg Se/kg (Gardiner, 1969). More recently, however, Whanger et al. (1978) reported WMD in lambs fed diets containing .1 mg Se/kg. The reasons for these disparate reports are not apparent, but they point to the existence of nutritional or environmental factors that can have a profound effect on Se requirement.

High levels of oral Cu have been reported to reduce Se availability in nonruminants (Hill, 1974.; Jensen, 1975; Stowe and Brady, 1980; Van Vleet et al., 1981). Copper often is added to mineral mixes for ruminants, and Cu toxicity is relatively common in sheep (Underwood, 1981). The aim of the following work was to test the hypothesis that dietary Cu, alone or in combination with Mo, reduces Se status in sheep. Molybdenum was included as a treatment because of the known interaction involving Mo and Cu (Dick et al., 1975; Mills et al., 1978) and because of the possibility that Mo and Cu combines with Se in the rumen to form insoluble Cu selenomolybdates (Müller and Diemann, 1968). Interactions involving Se and thiomolybdates have been demonstrated in the rat, where ammonium tetrathiomolybdate in the drinking water resulted in a marked decrease in liver activity of GSHpx (White et al., 1985).

### Experimental Procedure

**Treatments and Diets.** At 1 mo prior to breeding, 36 mature Hampshire ewes were divided equally into four treatment groups and offered the following salt-mineral mixtures ad libitum: 1) basal, 2) basal plus .12% Cu, 3) basal plus .12% Mo and 4) basal plus .12% Cu plus .12% Mo. The analyzed level of minerals naturally present in the diet and the composition of the mineral mix are shown in Table 1. Copper was added as cupric carbonate and Mo as Na molybdate. The concentration of .12% Cu and Mo provided approximately 10 mg/kg of each element in the total diet; daily mineral mixture intake averaged 16 g with a total dry feed intake of 2.0 kg. Ewes remained on treatments until their lambs were weaned at 10 wk postpartum. From 1 mo prior to mating, all ewes were fed to maintenance on alfalfa hay (full bloom). During a 1-mo breeding season, the ewes were run together with a Suffolk ram. Two weeks prior to completion of mating, and for the remainder of the experiment, ewes were fed a legume-grass low-moisture silage (15.4% CP). At 2 wk prepartum, they were fed an additional 230 g/d grain supplement (corn:oats, 3:1). After lambing, this was increased to 454 g/d. Lambs were given the same level of Cu and Mo supplement as their dams. This was supplied at 10 mg/kg in their creep feed (Table 1) because they were unable to reach the box containing the mineral mixture. The creep feed was offered to the lambs from birth and was inaccessible to the ewes.

**Sampling and Analysis.** Blood was sampled by jugular venipuncture from the ewes at five times during the experiment: once prior to treatment, twice during pregnancy (wk 9 and 17) and twice after parturition (3 d postpartum and at weaning). Lambs likewise were sampled at 3 d postpartum and at 2, 4, 5, 6, 8 and 10 wk (weaning). Lambs were weighed at the 3 d sampling and at weaning.

Blood was taken into 10-ml syringes containing 1 drop of heparin solution (3,000 lithium heparin IU/ml in isotonic saline). Aliquots of whole blood were analyzed for packed cell volume (hematocrit), hemoglobin (Crosby et al., 1954) and Se (Hoffman et al., 1968). The remaining blood was centrifuged (15 min at 750 × g) and plasma was removed. Packed cells were washed twice with isotonic saline containing phosphate buffer (.05 M, pH 7.4) and hemolyzed by adding an equal volume of cold distilled water. The hemolysate was assayed for GSHpx (using H2O2 as substrate) by the method of Paglia and Valentine (1967). One enzyme unit represents
1 μ mol NADPH oxidized per min at 25°C. Plasma was assayed for Cu, after dilution with distilled water (1:1), by direct aspiration into the flame of an atomic absorption spectrophotometer. Copper concentration was calculated using aqueous standards containing 10% glycerol.

At wk 5 and 10, five lambs from each group were selected to obtain an equal distribution of sexes between groups and killed. Liver and kidney were analyzed for Cu, Se and GSHpx. Glutathione peroxidase activity was determined on supernatant fluids from tissue homogenates (1:20 w/v) in .02 M phosphate buffer by the method of Lawrence et al. (1974).

Copper analysis of feed and tissues was by flame atomic absorption spectroscopy. Two grams of wet tissue or dried (105°C for 24 h), ground feed material were digested in nitric-perchloric-sulfuric acid (7:2:3). Selenium analysis was by the method of Hoffman et al. (1968) as modified by Oh et al. (1974). National Bureau of Standards (U.S. Dept. of Commerce, Washington, DC) bovine liver was included as an external standard for quality control in both Se and Cu assays.

Sulfur and Mo assays on feeds were carried out by the University of Wisconsin Soil and Plant Analysis Laboratory using emission spectroscopy (Schulte et al., 1987).

Statistical Analyses. Data were analyzed by ANOVA and linear regression (Steel and Torrie, 1960). Duncan’s multiple-range tests were applied when ANOVA indicated a significant (P < .05) treatment response (Steel and Torrie, 1960).

Results and Discussion

Selenium. In the ewes, GSHpx activity and Se concentration in blood declined during pregnancy and lactation, but Cu and Mo supplements had no effect on either criterion (Figure 1A,B). It was notable that there was little decrease in either Se or GSHpx values up to 4 wk prepartum, despite the replacement of the alfalfa hay (.1 mg/kg Se) with the silage (.05 mg/kg Se) at mating (wk -21). However, between the 4 wk prepartum and 3 d postpartum sampling, blood Se decreased by 60%, whereas erythrocyte GSHpx activity fell by 40%. This represents a minimal half-time for GSHpx in erythrocytes of approximately 35 d, a rate considerably faster than red cell turnover (half-time, 60 d). Oh et al. (1976) reported a similar marked drop in blood Se concentration during lactation in Hampshire ewes, but no decrease prior to parturition. In the current experiment, it is likely that the change from a high-Se diet of alfalfa hay to the low-Se silage at mating contributed to a decline in blood Se during gestation, and that the onset of lactation exacerbated this decrease. Whatever the reason, the results show that a .05 mg Se/kg diet was insufficient to maintain the Se status of the ewe in the final stages of gestation or the initiation of lactation.

For lambs, there were no effects of Cu or Mo on the activity of GSHpx or on the concentration of Se in whole blood (Figure 2A, B) or tissues (Table 2). Erythrocyte GSHpx activity in lambs at 3 d postpartum was correlated positively with activity in ewes at this time, although newborn lambs had higher blood enzyme activities than their dams. The regression was: Y[lamb] = 1.027X[ewe] + .048; r = .66 (P < .05). Selenium concentration in whole blood was correlated positively with erythrocyte GSHpx activity (r = .80) and values for GSHpx and Se in blood of lambs declined by over 30% during the preweaning period (P < .05), with the greatest fall in the first 5 to 6 wk (Figure 2). These levels, although not markedly deficient, are in the upper range in which WMD is commonly encountered (ARC, 1980). This, combined with the decline in liver GSHpx, provides further evidence that .05 mg Se/kg diet is insufficient for maintaining optimal Se status in breeding ewes and their lambs.

Copper. For ewes, Mo treatment decreased plasma Cu, the effect being most obvious at parturition (Figure 3). Molybdenum-supplemented ewes had the same level of plasma Cu as other groups at the sampling prior to parturition, but they did not show the postpartum increase seen in other groups. This suggests that there were abnormalities in Cu metabolism, although plasma Cu concentrations remained within the accepted normal range of .8 to 1.2 mg/litter (Underwood, 1981). According to Mills et al. (1978), Mo, at 2 to 5 mg/kg in the diet, reduced plasma Cu, whereas at 15 to 50 mg/kg, it increased plasma Cu but

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7Model 403, Perkin-Elmer Corp., Norwalk, CT.
at 15 to 50 mg/kg, it increased plasma Cu but decreased liver Cu. Thus, the response of plasma Cu to excess Mo can be variable and depends on the level of dietary S as well as the level of Cu in the liver at the start of treatment. Certainly the transfer of Cu to lambs of Mo-supplemented ewes was inhibited (see below), and there is little doubt that a continued supply of supplementary Mo would have resulted in a Cu deficiency in the ewes. The observed increase in plasma Cu concentrations after parturition has been reported by others and apparently is a normal phenomenon in sheep (Butler, 1963).

Plasma Cu concentrations in the ewe at parturition gave a reliable prediction of those in lambs at birth: \[ Y_{[lamb]} = 0.81X_{[ewe \ Cu]} - 0.13; \ r = 0.95, \ P < 0.001. \] Copper concentrations in plasma of newborn lambs from Mo-supplemented ewes were 28% lower \((P < 0.05)\) than from ewes fed other diets (Figure 4). Copper and Mo treatments had a marked effect on the Cu concentration in liver of lambs (Figure 5). However, there was no evidence of Cu toxicosis, such as reduced growth or hemolytic crisis, and neither was there any evidence of Cu deficiency, such as anemia. Growth rate averaged 280 g/d, and hemoglobin concentrations were not affected by treatments (Figure 6). Despite the absence of clinical manifestations, however, the liver Cu concentrations from the lambs on the Mo- and Cu-
TABLE 2. SELENIUM CONCENTRATION AND GLUTATHIONE PEROXIDASE (GSHpx) ACTIVITY IN LIVER AND KIDNEY OF LAMBS AT 5 AND 10 WK OF AGE (RESULTS ARE MEAN ± SE ON A FRESH-WEIGHT BASIS)

<table>
<thead>
<tr>
<th>Item</th>
<th>Se, mg/kg</th>
<th>GSHpx, EU/g</th>
<th>Se, mg/kg</th>
<th>GSHpx, EU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 wk</td>
<td></td>
<td>10 wk</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (C)</td>
<td>.09 ± .01</td>
<td>.41 ± .04</td>
<td>.13 ± .01</td>
<td>.27 ± .02</td>
</tr>
<tr>
<td>C + Cu</td>
<td>.09 ± .01</td>
<td>.37 ± .07</td>
<td>.10 ± .01</td>
<td>.28 ± .02</td>
</tr>
<tr>
<td>C + Mo</td>
<td>.10 ± .01</td>
<td>.38 ± .06</td>
<td>.11 ± .01</td>
<td>.26 ± .04</td>
</tr>
<tr>
<td>C + Cu + Mo</td>
<td>.10 ± .01</td>
<td>.37 ± .02</td>
<td>.13 ± .01</td>
<td>.28 ± .02</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (C)</td>
<td>.49 ± .02</td>
<td>.88 ± .22</td>
<td>.47 ± .07</td>
<td>nm</td>
</tr>
<tr>
<td>C + Cu</td>
<td>.40 ± .05</td>
<td>.89 ± .20</td>
<td>.50 ± .05</td>
<td>nm</td>
</tr>
<tr>
<td>C + Mo</td>
<td>.52 ± .02</td>
<td>.82 ± .20</td>
<td>.47 ± .05</td>
<td>nm</td>
</tr>
<tr>
<td>C + Cu + Mo</td>
<td>.55 ± .02</td>
<td>.78 ± .31</td>
<td>.50 ± .02</td>
<td>nm</td>
</tr>
</tbody>
</table>

*EU = enzyme unit.

*bNot measured.

Figure 2. Glutathione peroxidase activity (A) and Se concentration (B) in blood of lambs (mean ± SE).
supplemented treatments indicated that Cu deficiency and toxicosis, respectively, almost certainly would occur if the treatments continued after weaning. For example, lambs on Mo-supplemented diets showed a 50% reduction in liver Cu levels between 5 and 10 wk. The final level of 11 mg Cu/kg in the liver, combined with plasma mean level of just above .5 mg/kg at birth (Figure 4), indicated a marginal deficiency (Underwood, 1981). Likewise, the increase in liver Cu between 5 and 10 wk for the Cu-supplemented lambs indicated a continuing Cu accumulation that may result in Cu toxicosis.

Observations indicated that lambs ate almost none of the creep feed prior to the 5 wk sampling. Hence, results for the 5 and 10 wk samples indicated that preruminating lambs from Mo-supplemented ewes not only had reduced liver Cu levels at birth, but that transfer of Cu to the lamb via milk also was reduced. With increased intake of the creep feed after 5 wk, the availability of Cu was further reduced, presumably via the formation of Cu thiomolybdates in the rumen. Conversely, lambs from Cu-supplemented dams would be expected to have increased liver Cu at birth and receive a greater amount of Cu from the milk than lambs from the control or Mo-supplemented groups. After 5 wk, lambs would have received additional Cu from the creep feed, further increasing liver Cu concentration.

Unlike liver, kidney Cu concentrations were uniformly low and not affected by treatment.
Average values for 5 and 10 wk samples (mg/kg fresh weight) were 5.4 ± .3 and 3.8 ± .2, respectively.

In conclusion, the results showed no evidence that dietary supplements of Cu or Mo at 10 mg/kg affected either the availability of dietary Se to sheep, or the activity of GSHpx in blood, liver or kidney. This finding does not preclude effects at higher levels of Cu and Mo. However, in terms of practical diets for sheep, it would appear that the sporadic nature of outbreaks of Se-responsive diseases cannot be ascribed to the use of Cu or Mo supplements.

**Literature Cited**


