THE METABOLISM OF $^{75}$Se-SELENOMETHIONINE IN SHEEP GIVEN SUPPLEMENTARY COPPER AND MOLYBDENUM$^{1,2}$


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ABSTRACT

Four groups of three ram lambs were fed, in group pens, the following diets from 4 to 19 wk of age: 1) control (6 mg Cu/kg and 1 mg Mo/kg); 2) control plus 10 mg Cu/kg; 3) control plus 10 mg Mo/kg and 4) control plus 10 mg/kg of both Cu and Mo. Copper and Mo were added to the diet as copper sulfate and sodium molybdate. The main ingredients of the diets were alfalfa hay (20%), oats (20%) and corn (59%). At 19 wk, the animals were allocated randomly to individual metabolism cages and received a single oral dose of $^{75}$Se-selenomethionine. Liver Cu concentrations at slaughter (22 wk) were 77, 259, 68 and 316 mg/kg fresh weight for treatments 1 through 4. There was clinical evidence of Cu poisoning in lambs on treatment 2. Sheep given Cu supplements without additional Mo had reduced ($P < .05$) levels of $^{75}$Se activity in muscle compared with control animals. This decrease in muscle $^{75}$Se in Cu-supplemented lambs was associated with a nonsignificant increase in $^{75}$Se content of other tissues and a nonsignificant increase in fecal excretion of $^{75}$Se. Apparent absorption and net retention of $^{75}$Se was 80% and 74%, respectively. Long-term ingestion of moderate levels of Cu influenced the metabolism of Se fed as selenomethionine, possibly through effects of chronic Cu toxicity on liver function.

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


Introduction

Outbreaks of Se-responsive diseases are sporadic in terms of location and season (Underwood, 1981). This indicates the existence of other dietary factors that might influence Se availability. Perhaps Cu is such a factor. Copper deficiency and toxicosis are not uncommon in sheep. Barry et al. (1981) showed that calves made copper-deficient by feeding them kale had reduced concentrations of Se in blood. Likewise, in nonruminants, Se status is reduced either by feeding very high levels of dietary Cu (Hill, 1974; Jensen, 1975; Stowe and Brady, 1980; Van Vleet et al., 1981) or by Cu deficiency (Jenkinson et al., 1982; White et al., 1985). These effects of a Cu deficiency on reducing Se status in rats can be achieved either by feeding a low-Cu diet or by adding ammonium tetrathiomolybdate to the drinking water (White et al., 1985). Thiomolybdates are formed in the rumen in the presence of Mo and sulfide, and they reduce Cu absorption and systemic Cu availability in sheep (Mills et al., 1978).

In previous experiments, we showed that adding the equivalent of 10 mg Cu/kg or 10 mg Mo/kg to the diet of pregnant ewes had
either little or no effect on the Se status of the ewes or their lambs (White et al., 1979, 1989). The aim of the present experiment was to investigate whether long-term supplementation of lambs with similar levels of Cu or Mo alters the availability or metabolism of Se. $^{75}$Se-selenomethionine ($^{75}$Se-met) was used as the tracer because it is considered to be one of the primary sources of Se ingested by ruminants (Peterson and Butler, 1962; Jenkins and Hidiroglou, 1967).

**Materials and Methods**

**Animals and Treatments.** Four groups of 10 Hampshire x Suffolk crossbred lambs of mixed sex were offered the following dietary treatments: 1) control (basal diet); 2) basal plus 10 mg Cu/kg as Cu sulfate (plus-Cu); 3) basal plus 10 mg Mo/kg as sodium molybdate (plus-Mo); 4) basal plus 10 mg Cu/kg plus 10 mg Mo/kg (Cu-plus-Mo). The lambs were fed, in group pens with their dams, a creep basal diet from 4 to 9 wk of age. It consisted of 40% ground alfalfa hay, 48.5% ground shelled corn, 10% soybean meal, 1.0% dicalcium phosphate (24% Ca, 18.5% P) and .5% salt mixture. At weaning (9 wk), the lambs were offered a pelleted grower diet consisting of 20% alfalfa hay, 20% oats, 59.25% ground shelled corn, .5% mineral mix and .25% antibiotic-sulmet premix. The mineral mix was the same as that in the creep diet. The respective concentrations of Cu, Mo, Se and S in the basal diet (mg/kg DM basis) were 5.5, 1.2, .04 and 1,700. At weaning (9 wk), five lambs were slaughtered and their tissue were analyzed for Se and glutathione peroxidase (GSHpx). The results of this part of the experiment are summarized elsewhere (White et al., 1979).

At 19 wk of age, three ram lambs (average BW 52 kg) were selected randomly from each group and placed in wooden metabolism crates. Plastic urine collectors were attached with harnesses and glued around the prepuce. Feces bags (50 µm thick polyvinylchloride) were adhered directly to the wool. The sheep were fed 1,000 g of the respective treatment diet (12% moisture) in two equal parts at 0900 and 1600. Deionized water was available ad libitum. After a 7-d period of adjustment, the sheep were given by bailing gun a single gelatin capsule containing the $^{75}$Se-met, and fecal and urine collections were started.

**Preparation of $^{75}$Se-Selenomethionine.** The contents of six vials, each containing 275 µCi L-$^{75}$Se-met in aqueous solution (specific activity 10 µCi/µg Se), were mixed as a slurry with 4.49 g cellulose powder containing 12 mg Se as selenomethionine. The mixture was dried for 24 h at 70°C under vacuum. At weaning (9 wk), the lambs were offered a pelleted grower diet consisting of 20% alfalfa hay, 20% oats, 59.25% ground shelled corn, .5% mineral mix and .25% antibiotic-sulmet premix. The mineral mix was the same as that in the creep diet. The respective concentrations (mg/kg DM) of Cu, Mo, Se and S in the basal grower diet were 6.4, 1.2, .07 and 1,600. Copper and Mo were added at 10 mg/kg to the respective treatment diets as CuSO₄·5H₂O and Na₂MoO₄.

Fecal samples were collected daily for 7 d. Wool samples were taken with fine clippers and washed in hexane and ethanol before drying at 70°C under vacuum. Liver, kidney, testis and heart samples were taken at slaughter (d 13) and organs were weighed. The animals were killed by exsanguination under abattoir conditions. Samples of liver (ventral lobe), kidney (cortex and medulla), heart (left ventricle), testis and skeletal muscle (femoral) were homogenized immediately in ice cold distilled water (20% w/v) using a Polytron homogenizer. Counting was done directly on feces, urine, plasma, whole blood, wool, and tissue homogenates.

**Sampling and Analysis.** Blood samples were taken by jugular venipuncture into heparinized tubes at 4-h intervals for the first 24 h, daily for 7 d and, finally, at the terminal sampling (13 d after the dose). A sample of whole blood was taken for hemocrit measurement and radioactive counting. The remaining blood was centrifuged at 3,000 rpm for 15 min and the plasma was removed and stored at -20°C prior to assay and counting. Feces and urine were collected daily for 7 d. Wool samples were taken with fine clippers and washed in hexane and ethanol before drying at 70°C under vacuum. Liver, kidney, testis and heart samples were taken at slaughter (d 13) and organs were weighed. The animals were killed by exsanguination under abattoir conditions. Samples of liver (ventral lobe), kidney (cortex and medulla), heart (left ventricle), testis and skeletal muscle (femoral) were homogenized immediately in ice cold distilled water (20% w/v) using a Polytron homogenizer. Counting was done directly on feces, urine, plasma, whole blood, wool, and tissue homogenates.

Further aliquots of crude tissue homogenates were taken for analysis of Se, GSHpx and Cu. Glutathione peroxidase activity in supernatant fluid from tissues was assayed by the method of Lawrence et al. (1974); GSHpx in whole blood and plasma was assayed by the method of Paglia and Valentine (1967). An
### TABLE 1. ABSORPTION, EXCRETION AND RETENTION OF $^{75}$Se

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total accumulative $^{75}$Se activity, percentage of dose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Feces</th>
<th>Apparent absorption</th>
<th>Urine</th>
<th>Retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plus-Cu</td>
<td></td>
<td>17 ± 1</td>
<td>83 ± 1</td>
<td>6 ± 1</td>
<td>77 ± 1</td>
</tr>
<tr>
<td>Plus-Mo</td>
<td></td>
<td>21 ± 3</td>
<td>79 ± 3</td>
<td>7 ± 1</td>
<td>71 ± 4</td>
</tr>
<tr>
<td>Cu-plus-Mo</td>
<td></td>
<td>20 ± 2</td>
<td>80 ± 2</td>
<td>6 ± 1</td>
<td>74 ± 2</td>
</tr>
</tbody>
</table>

$^{a7}$-d collection; mean ± SE.

element unit (EU) represents 1 µmol NADPH oxidized per min at 25°C. Copper was determined using flame atomic absorption spectroscopy<sup>11</sup>. Tissue and feed samples were digested in a mixture of nitric/perchloric/sulfuric acid prior to assay. Diluted plasma was assayed by direct aspiration into the flame. The external Cu standard for tissue and feed was National Bureau of Standards<sup>12</sup> bovine liver and orchard leaves. Standard curves for plasma Cu assays were prepared from aqueous standards containing 10% glycerol. Ceruloplasmin in plasma was assayed using dimethyl-p-phenylenediamine in acetate buffer, pH 6.4 (Sunderman and Nomoto, 1970). Enzyme units are milligrams ceruloplasmin/liter.

Sulfur and Mo analyses of the diets were carried out by the University of Wisconsin Soil and Plants Analysis Laboratory. Selenium was analyzed in feed and tissues by the method of Hoffman et al. (1968) as modified by Oh et al. (1974).

Histological examination of liver samples was carried out with no prior knowledge of treatment.

**Statistical Analysis.** Statistical analysis was by ANOVA and linear regression (multivariate general linear hypothesis; least squares procedure) using the microcomputer program Systat<sup>13</sup>. Comparison among treatment means was by ANOVA and Duncan's Range Test (Steel and Torrie, 1980) using Systat on a completely randomized design. Animal was considered the experimental unit, so sources of variation for ANOVA and Duncan's Range Test were 3 degrees of freedom between groups and 8 degrees of freedom within groups. In addition to the range test, ANOVA by orthogonal contrasts was carried out to test for effects of Cu, Mo or Cu × Mo interaction. This involved decomposing the sum of squares among treatment groups into a set of orthogonal single degree of freedom comparisons. The two types of statistical comparisons (Duncans or orthogonal contrast) are considered equally legitimate ways of examining the same set of means and gave similar outcomes with two exceptions. These exceptions are discussed in the text.

### Results and Discussion

**$^{75}$Se-Retention.** Neither Cu nor Mo supplements had any significant effect on the absorption or retention of $^{75}$Se. The main excretory pathway for $^{75}$Se was the feces, and apparent absorption over the 7-d collection period averaged 80%, with 74% retention (Table 1). In the only comparable experiment using oral $^{75}$Se-met, Ehlig et al. (1967) reported an apparent absorption of between 60 and 70% for a 7-d collection, with net retention values between 40 and 50%. The higher values recorded in the current experiment could be attributed to the differences in dietary Se concentrations. Percentage Se retention is reduced at higher Se intakes (White and Somers, 1977), and Ehlig et al. (1967) fed a diet containing .3 mg Se/kg diet, compared with .04 mg/kg diet in the current experiment.

**$^{75}$Se in Blood.** The Cu-plus-Mo treatment reduced plasma radioactivity by 20% on d 1 compared with other treatments ($P < .05$; Figure 1). In addition, the plus-Mo treatment increased the amount of $^{75}$Se in red cells on d 1 ($P < .05$; Figure 2) compared with other treatments. Copper alone had no significant effects on blood or plasma radioactivity. The mean biological half-life for $^{75}$Se activity in plasma was 12 d for the period d 1

<sup>11</sup>Model 403, Perkin-Elmer Corp., Norwalk, CT.
<sup>12</sup>NBS; U.S. Dept. of Commerce, Washington, DC.
<sup>13</sup>Systat Inc., Evanston, IL.
Figure 1. Radioactivity of $^{75}$Se in total blood plasma (estimated as 6% live weight). Values are means ± SEM. The Cu-plus-Mo treatment means were less ($P < .05$) than other treatments.

Figure 2. Radioactivity of $^{75}$Se in total body packed cells. Values are means ± SEM ($n = 3$) calculated by difference from plasma and whole blood counts. Values for the plus-Mo treatment were higher ($P < .05$) than for other treatments.
TABLE 2. $^{75}$Se IN BLOOD AND TISSUES SAMPLED 13 DAYS AFTER DOSING

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Muscle a</th>
<th>Blood b</th>
<th>Liver</th>
<th>Kidney (2)</th>
<th>Testes (2)</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.9 c</td>
<td>6.0</td>
<td>5.0</td>
<td>3.2</td>
<td>2.3</td>
<td>.8</td>
</tr>
<tr>
<td>Plus-Cu</td>
<td>11.6 d</td>
<td>7.1</td>
<td>5.5</td>
<td>4.0</td>
<td>3.1</td>
<td>.8</td>
</tr>
<tr>
<td>Plus-Mo</td>
<td>13.2 c,d</td>
<td>8.1</td>
<td>5.3</td>
<td>3.5</td>
<td>2.8</td>
<td>.7</td>
</tr>
<tr>
<td>Cu-plus-Mo</td>
<td>13.6 c,d</td>
<td>6.0</td>
<td>5.9</td>
<td>3.7</td>
<td>2.8</td>
<td>.7</td>
</tr>
<tr>
<td>SE</td>
<td>1.3</td>
<td>.7</td>
<td>.4</td>
<td>.3</td>
<td>.6</td>
<td>.1</td>
</tr>
</tbody>
</table>

aMuscle weight calculated as 28% of live weight (Kempster and Cuthbertson, 1977)
bBlood weight calculated as 6% of live weight.
c,dMeans in the same column with different letters in their superscripts differ ($P < .05$).

There were no significant effects of treatment on organ weight (data not shown). Skeletal muscle contained the highest portion of the $^{75}$Se at d 13 (Table 2). Supplementation with Cu alone decreased ($P < .05$; Duncan’s range test) total radioactivity in muscle. This represented a difference of 5.3% of the dose retained, and accounted for most of the nonsignificant decrease in retention by the plus-Cu group. Supplementation with Mo alone or with Mo plus Cu resulted in intermediate muscle concentrations.

Kidneys contained more than 10-fold the concentration of $^{75}$Se of other tissues (counts/weight) and totalled .26% of live weight. Heart contained a slightly higher proportion of the dose relative to organ size (.5% of live weight), whereas skeletal muscle at 28% of live weight contained only 14% of the dose. There were no significant effects of treatment on total $^{75}$Se activity in individual tissues other than skeletal muscle.

A possible explanation for the decrease in muscle $^{75}$Se in the Cu-supplemented sheep is that the liver damage caused by excess Cu was influencing the metabolism and, hence, altering the distribution of absorbed $^{75}$Se-selenomethionine. This conclusion is supported by the fact that Cu reduced the specific activity of $^{75}$Se in muscle (see below). However, there remains some doubt as to the biological significance of this because orthogonal contrast ANOVA showed only a $P = .1$ for the effect of Cu on total $^{75}$Se activity in muscle. It could be argued that the effect is marginal and dependent on the degree of Cu toxicosis, but the small number of animals per group makes it difficult to be certain. Selenium concentration in sheep liver has been shown to be increased due to either acute Cu toxicosis (Gooneratne and Howell, 1981) or fungal toxins (Allen et al., 1979). The latter workers showed that sheep affected by lupinosis toxin had increased concentrations of Cu and Se in the liver, and that liver damage was correlated directly with increased liver Cu and Se. In the current experiment, the Cu supplement had an effect on the liver, as demonstrated by two animals on the plus-Cu treatment having hemolytic crises just prior to the start of the radiotracer experiment (19 wk). One of these animals died; the other recovered but was omitted from the tracer experiment. Of the three sheep selected, one in the plus-Cu group showed histological evidence of chronic Cu toxicity in the liver at slaughter. The liver contained swollen necrotic cells, primarily Kupffer cells, with scattered clusters of neutrophils. No sheep in other groups showed evidence of Cu toxicity, despite high liver Cu concentrations in animals in the Cu-plus-Mo group. The appearance of Cu toxicosis in
Figure 3. The concentrations of Se and 75Se and the activity of glutathione peroxidase (GSHpx) in tissues (wet weight) and blood. Values are means ± SEM for combined treatments (N = 12).

sheep fed diets of 16 mg Cu/kg is not unusual. Housed lambs appear to be especially susceptible, and Adamson et al. (1969) reported chronic Cu toxicosis in lambs fed diets containing less than 20 mg Cu/kg diet for 27 wk. Total dietary S was not reported but Mo concentration was under 1 mg/kg and sulfate-S was less than 1 g/kg. A dietary copper level of only 16 mg/kg DM therefore is dangerous to Hampshire × Suffolk lambs reared indoors when dietary S and Mo concentrations are in a range typically encountered with forage-based diets.

Although Mo addition at 10 mg/kg prevented the appearance of clinical signs of Cu toxicity, it did not reduce the concentration of Cu in liver (P > .05; Table 3). Orthogonal contrast ANOVA indicated significant effects of Cu treatment on liver Cu concentration (P < .05) but no effects of Mo and no interaction between Cu and Mo (P > .05). On the basis of results of Suttle (1974), this lack of effect of Mo on liver Cu was unexpected. However, in hindsight the poor response probably was due to the low dietary S concentration (1.6 g/kg), which is below that required for the maintenance of high ruminal sulfide levels (Bray and Hemsley, 1969) and, hence, for the formation of insoluble Cu-thiomolybdate complexes (Grace and Suttle, 1979). Wittenberg and Devlin (1987) showed almost no effect of adding even 20 mg Mo/kg DM on plasma Cu concentration in steers when dietary S concentration was low (1.3 mg/kg), and certainly, ruminal sulfide plays a major part in the relative effectiveness of Mo on Cu absorption in ruminants (Suttle, 1983). At an S level of 1.6 mg/kg, Mo concentration would be expected to increase in blood and tissues (Kincaid and White, 1988). This may have contributed to the effects of Mo on 75Se metabolism in blood.

The liver from one animal in the plus-Mo group had marked periportal fatty change extending about a third of the way into the lobule. This was accompanied by moderate accumulation of periportal lymphocytes and slight bile duct epithelial hyperplasia with no increase in connective tissue. There are no data suggesting that Mo supplements cause microscopic liver damage, so the etiology of this condition was unknown. Feed intakes were not affected greatly by treatment; all sheep ate the 1 kg/d offered with the exception of one sheep (Cu-plus-Mo) that ate, on average, 813 g, and one sheep (Plus Cu) that ate 928 g. Dry matter digestibility was not affected by treatment and averaged 79% for the 7-d fecal collection.

Relationship between Se, 75Se and GSHpx.

With one exception, Se concentration and GSHpx activity in blood and tissues were not affected by treatment, although there were large differences among tissues (Figure 3). The exception was a decrease in plasma GSHpx activity due to Mo (orthogonal contrast; P < .05); with values of .13 EU/ml for plus-Mo treatments and .21 EU/ml for treatments

### TABLE 3. EFFECT OF TREATMENT ON COPPER STATUS OF SHEEP (MEAN ± SE)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver Cu, mg/kg fresh</th>
<th>Plasma Cu, mg/liter</th>
<th>Plasma ceruloplasmin, mg/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77 ± 11a</td>
<td>.91 ± .11</td>
<td>532 ± 33</td>
</tr>
<tr>
<td>Plus-Cu</td>
<td>241 ± 19b</td>
<td>1.30 ± .03</td>
<td>503 ± 78</td>
</tr>
<tr>
<td>Plus-Mo</td>
<td>68 ± 14b</td>
<td>1.09 ± .15</td>
<td>573 ± 44</td>
</tr>
<tr>
<td>Cu-plus-Mo</td>
<td>316 ± 31b</td>
<td>1.13 ± .17</td>
<td>420 ± 64</td>
</tr>
</tbody>
</table>

a,bMeans in the same column with different letters in their superscripts differ (P < .05).
Figure 4. The relationship between the activity of ceruloplasmin and glutathione peroxidase (GSHpx) in plasma. The regression equation is CPN = 1,616 \times \text{GSHpx} + 170; R^2 = .67, SE(slope) = 357, SE (intercept) = 77 and P = .001.

without Mo. This result supports those in Figure 1, whereby Mo reduced plasma $^{75}$Se concentration.

$^{75}$Se labeling of tissues closely paralleled Se concentration, demonstrating that oral dosing with $^{75}$Se-met results in tissue labeling that is similar to the concentration of Se achieved by feeding natural dietary ingredients. In contrast with Se, GSHpx activity did not correlate with $^{75}$Se concentration in tissues (Figure 3). Kidney contained the greatest $^{75}$Se activity and Se concentration, and heart and blood had the highest activity of GSHpx. Thus, a large proportion of Se in liver and kidney must exist in forms other than GSHpx, and Se from selenomethionine must be incorporated into different compounds in different tissues.

There were significant differences among tissues with respect to specific radioactivity; kidney had the highest activity, whereas skeletal and heart muscle had the lowest activity ($P < .05$; Table 4). These differences presumably reflect differences in $^{75}$Se turnover rate. The plus-Cu treatment lowered the specific radioactivity of muscle ($P < .05$) and tended to reduce the specific radioactivity in blood, kidney, testis and heart ($P > .05$).

There was no significant correlation ($P > .05$) between ceruloplasmin activity in plasma and Cu concentration in liver or plasma. Others have reported a relatively tight relationship between plasma Cu and ceruloplasmin activity in ruminants (Amer et al., 1973; Osman et al., 1984). However, this relationship is not apparent when the range of values for Cu concentration is small (Kincaid et al., 1986), and the correlation disappears altogether when Mo intakes are high (Mills et al., 1981). In the current experiment, the sheep apparently were normal with regard to Cu status, and the range of plasma Cu concentrations was small. It is, therefore, not surprising that Cu concentration in liver and plasma did not correlate significantly with ceruloplasmin activity. Perhaps of greater biological significance was the positive correlation between plasma ceruloplasmin activity and plasma GSHpx activity (Figure 4). This association was independent of treatment and suggests a possible relationship between ceruloplasmin and Se. Amer et al. (1973) claimed that .7 ppm

<table>
<thead>
<tr>
<th>Item</th>
<th>Muscle</th>
<th>Blood</th>
<th>Liver</th>
<th>Kidney</th>
<th>Testis</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.96</td>
<td>1.46</td>
<td>1.60</td>
<td>2.09</td>
<td>1.71</td>
<td>1.23</td>
</tr>
<tr>
<td>Plus-Cu</td>
<td>1.01</td>
<td>1.13</td>
<td>1.81</td>
<td>1.92</td>
<td>1.54</td>
<td>.98</td>
</tr>
<tr>
<td>Plus-Mo</td>
<td>1.32</td>
<td>2.19</td>
<td>1.94</td>
<td>2.23</td>
<td>2.10</td>
<td>1.41</td>
</tr>
<tr>
<td>Cu-plus-Mo</td>
<td>1.38</td>
<td>1.31</td>
<td>2.01</td>
<td>2.05</td>
<td>1.88</td>
<td>1.27</td>
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<tr>
<td>SE</td>
<td>.13</td>
<td>.31</td>
<td>.19</td>
<td>.31</td>
<td>.25</td>
<td>.15</td>
</tr>
</tbody>
</table>

Orthogonal contrast

| Effect of Cu | *  | NS    | NS    | NS     | NS     | NS    |
| Effect of Mo | NS | NS    | NS    | NS     | NS     | NS    |
| Effect of Cu x Mo | NS | NS    | NS    | NS     | NS     | NS    |

Mean organ specific radioactivity

$^{ab}$Orthogonal contrast anova. NS = not significant ($P > .05$).

$^{b}$Average specific radioactivity for each organ for all treatments.

$^{c}$Values in the same row with different letters in their superscripts differ ($P < .05$).
supplemental Se reduced ceruloplasmin synthesis in calves. An inspection of their data revealed an effect at only one time period, and so generalized conclusions about such an interaction probably were not warranted. In the current experiment, the range of values for Se and ceruloplasmin were small; this would increase the probability of a significant relationship occurring by chance. This relationship thereafter needs further investigation.

In summary, chronic ingestion of moderate levels of Cu and/or Mo supplements influenced the metabolism of Se given orally as $^{75}\text{Se}$-met. The main effect was small, but Cu supplements decreased $^{75}\text{Se}$ in muscle of sheep. This effect of Cu appeared to be indirect, perhaps as a result of liver damage associated with chronic Cu toxicity, and interactions involving Cu, Se, Mo and S are likely to affect the results. The effects of Mo on $^{75}\text{Se}$ metabolism were small in absolute terms and confined to the blood. No obvious mechanisms for the Mo effects were apparent from the results.

**Literature Cited**


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