Zinc and copper status in ewes supplemented with sulfate- and amino acid-complexed forms of zinc and copper


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ABSTRACT: Thirty 6-yr-old Targhee ewes were randomly allotted to one of five supplemental treatments to evaluate supplementation effects on liver and fecal Zn and Cu concentrations and serum alkaline phosphatase activity: 1) control, 2) Zn complex, 3) Zn and Cu (ZnCu) complex, 4) Zn sulfate, and 5) ZnCu sulfates. Supplements were administered daily in gelatin capsules for 56 d. Liver biopsies and serum samples were collected every 14 d starting on d 0. Supplemental Zn and Cu levels were formulated to provide 90 mg/kg Zn and 10 mg/kg Cu, respectively, on a daily dry matter intake basis. Form (complex vs sulfate) × type (Zn vs ZnCu) interactions were not detected (P > 0.35). Therefore, contrast statements were used to make the following treatment comparisons: 1) control vs supplement, 2) Zn vs ZnCu, and 3) complex vs sulfate. Ewe BW at the end of the study (P = 0.09) and ewe BW change from beginning to end of the study (P = 0.07) were greater for supplemented than control ewes. Body weight and BW change did not differ between sulfate and complex (P > 0.39) or Zn- and ZnCu- (P > 0.40) supplemented ewes. Liver Cu concentrations did not differ (P = 0.41) between control and supplemented ewes. Liver Cu concentrations were higher (P < 0.10) for ewes supplemented with ZnCu than Zn and complex than sulfate forms of supplement. Liver Zn concentration tended (P = 0.13) to be higher in ZnCu than Zn-supplemented ewes. Liver and fecal Zn concentration were higher (P < 0.06) in ewes fed complex than sulfate supplements. However, serum alkaline phosphatase activity tended (P = 0.12) to be greater in ewes fed sulfate than complex supplements. Supplementing mature ewes with complexed minerals resulted in higher concentrations of Zn and Cu in the liver. In addition, supplemental Cu tended to increase concentrations of Zn in the livers of ewes; however, high levels of supplemental Zn did not negatively impact liver Cu concentrations.

Key Words: Copper, Copper Sulfate, Sheep, Zinc, Zinc Sulfate

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Introduction

Zinc and copper are essential trace minerals, whose requirements, form (chelate, complex, sulfate, oxide, etc.), and interactions with each other are not clearly understood. Hatfield et al. (1992) demonstrated the benefit of feeding Zn at six times the NRC (1985) recommended level to feedlot lambs in a stressful environment. High levels of supplemental Zn in the form of Zn methionine were also shown to have a positive influence on ewe milk production and ultimately lamb weaning weights (Hatfield et al., 1995). In addition, sheep producers often feed supplemental Zn at concentrations higher than NRC (1985) recommendations to prevent foot infections in sheep subject to damp, muddy, confinement situations. However, the influence of form of supplemental Zn (organic vs inorganic) on sheep Zn status has not been well documented. In addition, Wellington et al. (1998) stated that caution must be exercised when supplementing excess Zn without Cu to avoid compromising the animal’s Cu status. Because Zn and Cu compete for binding sites on enzymes and metalloproteins, it may be reasonable to speculate that these minerals are antagonistic to some degree. Therefore, our objective was to evaluate the effect of high levels of complex and sulfate forms of Zn, with or without Cu, and the interactions of these factors and time on measures of Zn and Cu status in mature ewes.

Materials and Methods

Thirty 6-yr-old Targhee ewes (initial BW = 61 ± 1.9 kg) were randomly allotted to one of five supplemental
treatments: 1) control (no supplemental Zn or Cu), 2) 1.404 g of Availa-Zn 100 (complex metal amino acid with 10% Zn and 20% total amino acids, Zinpro Corp., Eden Prairie, MN, IFN 6-32-053), 3) 1.404 g of Availa-Zn 100 combined with 0.156 g of Availa-Cu 100 (complex metal amino acid with 10% Cu and 20.6% total amino acids, Zinpro Corp., IFN 6-32-053), 4) 0.386 g of Zn sulfate (ZnSO₄, 36.36% Zn, IFN 6-05-555, and 5) 0.386 g of Zn sulfate combined with 0.061 g of Cu sulfate (CuSO₄, 25.54% Cu, IFN 6-01-720). Treatment designations are as follows: 2 and 3 = complex, 4 and 5 = sulfate, 2 and 4 = Zn, and 3 and 5 = ZnCu. Trace mineral supplements were administered daily between 1600 and 1800 in gelatin capsules for 56 d. Supplements were formulated to provide 90 and 10 mg/kg DM daily, respectively, of Zn and Cu. Forage plus mineral supplements provided 102.2 and 15.4 mg/kg DM of Zn and Cu, which was approximately 3 times the Zn and 1.5 times the Cu recommended levels (NRC, 1985). Ewes were managed as one group in an outdoor pen and had ad libitum access to white salt and water. Ewes were group-fed between 0600 and 0800 kg/d or approximately 1.56 kg-ewe⁻¹-d⁻¹ long stem alfalfa hay (Table 1) and were weighed at the beginning and end of the study after an overnight shrink without food or water.

Before the study, the hay was core-sampled; every other bale was cored. Samples were composited, dried for 48 h at 60°C, ground through a 1-mm screen, and analyzed for CP, DM, Cu, Fe, Mo, S, and Zn (AOAC, 1999). Water samples were collected at the beginning, middle, and end of the study and were composited and analyzed for Cu, Fe, Mo, S, and Zn (AOAC, 1999).

To determine the effect of treatments on liver Zn and Cu status, liver biopsies and serum samples were collected on d 0, 14, 28, 42, and 56. Liver biopsies were performed under aseptic conditions using a Tru Cut biopsy needle (LVWR Scientific Products Corp., Seattle, WA) to collect 40 to 60 mg of hepatic tissue. A 10-cm² area encompassing the biopsy site was completely clipped of wool using electric clippers fitted with a surgical-grade blade attachment and locally anesthetized with 1 mL of lidocaine (20 mg/mL; Lido-epi, Radix Labs, Eau Claire, WI). A sample was obtained through a puncture incision made between the 11th and 12th ribs on the perceptual line from the tuber coxae to the point of the shoulder. Samples were physically separated from contaminating blood, placed in a 12 × 75-mm disposable culture tube (Fisher Scientific, Houston, TX), capped, and frozen at −20°C until overnight shipping for elemental analysis. Samples were sent to the Michigan State University Animal Health Diagnostic Laboratory, toxicology section (Lansing, MI). Zinc and Cu concentrations were determined by inductively coupled plasma-atomic emission spectroscopy with ultrasonnic nebulization as described by Braselton et al. (1997).

Treatment effects on concentrations of Zn and Cu in feces were determined by collecting fecal grab samples between 1600 and 1800 on d 52, 54, and 56. Samples were dried in a forced-air oven (50°C) and ground through a 1-mm screen. Equal weight samples representing each day’s collection were composited by ewe and analyzed for Zn and Cu by atomic absorption spectroscopy (AOAC, 1999).

Serum alkaline phosphatase activity was also measured using an alkaline phosphatase kit (Sigma Diagnostics, St. Louis, MO) as an indication of Zn status. Blood was collected from each ewe on d 0, 14, 28, 42, and 56 via jugular venipuncture using unheparinized Vacutainers (9.5 mL, Fisher Scientific, Houston, TX). Blood was allowed to coagulate at room temperature. Samples were centrifuged for 20 min at 1000 × g, separating serum from red blood cells. Serum was then decanted off into 12 × 75-mm plastic serum tubes, capped and stored frozen at −20°C until analyzed for alkaline phosphatase (Bessey et al., 1946). Results are presented as sigma unit of phosphatase activity, which is defined as the amount of enzyme activity that will liberate 1 μmol of p-nitrophenol/h under the test conditions described by Bessey et al. (1946).

Ewe was the experimental unit in a completely randomized block design. The repeated measures procedure of GLM (SAS Inst. Inc., Cary, NC) was used to evaluate liver Zn and Cu status, and alkaline phosphatase activity. Ewe BW and fecal concentrations of Zn and Cu were evaluated using the PROC GLM procedure (SAS Inst. Inc.). All models included the following contrast statements: 1) control vs supplemented, 2) complex vs sulfate, 3) Zn vs ZnCu, and 4) the interaction of supplement type (Zn and ZnCu) × supplement form (complex and sulfate). Initial concentrations of liver Zn and Cu, and initial serum alkaline phosphatase activity were included as covariables for the evaluation of liver Zn, Cu, and serum alkaline phosphatase activity, respectively.

Results

No supplement type × form interactions were detected (P > 0.41). In addition, no time or time × treatment interactions were detected (P > 0.30) except for time × Zn vs ZnCu (P = 0.08). Therefore, results, where appropriate, are presented as main effects of treatment (control, supplement form, and supplement type) average over d 14 to 56 of supplementation.

### Table 1. Hay and water mineral (and hay CP) composition

<table>
<thead>
<tr>
<th>Item</th>
<th>Hay</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>11.7</td>
<td>—</td>
</tr>
<tr>
<td>Cu, ppm</td>
<td>5.37</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fe, ppm</td>
<td>81.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mo, ppm</td>
<td>1.0</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>S, ppm</td>
<td>900</td>
<td>9.2</td>
</tr>
<tr>
<td>Zn, ppm</td>
<td>12.2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*On a DM basis.*
Table 2. Effects of form of supplemental Zn with or without Cu on BW and BW change during the 56-d study\textsuperscript{ab}

<table>
<thead>
<tr>
<th>Item</th>
<th>Control\textsuperscript{c}</th>
<th>Zn</th>
<th>ZnCu</th>
<th>Zn</th>
<th>ZnCu</th>
<th>SE</th>
<th>Control vs supplemented</th>
<th>Zn vs ZnCu</th>
<th>Complex vs sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final BW</td>
<td>59.2</td>
<td>60.4</td>
<td>67.4</td>
<td>65.1</td>
<td>62.2</td>
<td></td>
<td>0.09</td>
<td>0.40</td>
<td>0.93</td>
</tr>
<tr>
<td>BW change</td>
<td>0.3</td>
<td>1.7</td>
<td>2.5</td>
<td>2.9</td>
<td>3.1</td>
<td>1.05</td>
<td>0.07</td>
<td>0.61</td>
<td>0.39</td>
</tr>
</tbody>
</table>

\textsuperscript{a}No supplement type (Zn and ZnCu) × form (complex and sulfate) interaction (\(P > 0.35\)).

\textsuperscript{b}Supplements were formulated to provide 90 and 10 mg/kg DM daily, respectively, of Zn and Cu.

\textsuperscript{c}Control = no supplemental Zn or Cu.

\textsuperscript{d}Complex = Availa-Zn 100 (complexed metal amino acid with 10% Zn and 20% total amino acids) and Availa-Cu 100 (complexed metal amino acid with 10% Cu and 20.6% total amino acids).

\textsuperscript{e}Sulfate = Zn sulfate (36.36% Zn) and Cu sulfate (25.54% Cu).

Ewe BW at the end of the study (\(P = 0.09\)) and ewe BW change from beginning to end of the study (\(P = 0.07\)) were greater for supplemented than for control ewes (Table 2). Body weight and BW change did not differ between ewes supplemented with sulfate vs complex or Zn vs ZnCu (\(P > 0.39\)).

Liver Cu concentrations did not differ (\(P = 0.41\)) between control and supplemented ewes (Figure 1). A time \(\times\) Zn vs ZnCu interaction was detected (\(P = 0.08\)) for liver Cu concentration. Liver Cu concentrations remained relatively constant for Zn-supplemented ewes during the course of the study. During the same period, liver Cu concentrations for ZnCu-supplemented ewe increased 17%. Liver Cu concentration was higher (\(P = 0.10\)) in complex- than sulfate-supplemented ewes. Fecal Cu concentration was higher (\(P = 0.04\)) for supplemented than control ewes and was also higher (\(P = 0.001\)) for ZnCu- than Zn-supplemented ewes (Table 3). Fecal Cu concentration did not differ (\(P = 0.51\)) between complex- and sulfate-supplemented ewes.

Supplemented ewes had higher (\(P = 0.006\)) concentrations of liver Zn than control ewes (Table 4). In addition, liver Zn concentration tended (\(P = 0.13\)) to be higher in ZnCu- than Zn-supplemented ewes. Liver Zn concentration was higher (\(P = 0.06\)) in complex- than sulfate-supplemented ewes. Fecal Zn concentration was higher (\(P = 0.001\)) in supplemented than control ewes (Table 3) and tended to be higher (\(P = 0.18\)) in Zn than ZnCu-supplemented ewes. Fecal Zn concentration was higher (\(P = 0.01\)) in ewes given the complex than in those given the sulfate form.

Serum alkaline phosphatase activity did not differ (\(P = 0.81\)) between control and supplemented ewes (Table 4). Alkaline phosphatase activity was greater (\(P = 0.05\)) in Zn- than ZnCu-supplemented ewes. In addition, alkaline phosphatase activity tended (\(P = 0.19\)) to be greater in sulfate- than complex-supplemented ewes.

**Discussion**

**Ewe Body Weight**

The hay used in our study had low Zn (12.2 mg/kg DM), low Cu (5.37 mg/kg DM), and 1 mg/kg DM Mo. The NRC (1985) suggests 33 mg Zn/kg DM for maintenance and 8 to 11 mg Cu/kg DM in the diet for ewes when Mo is less than 1 mg/kg DM. Supplemented ewes gained more BW than control ewes. Ewe BW change did not differ between Zn- and ZnCu-supplemented ewes. However, complex ZnCu- and sulfate ZnCu-supplemented ewes gained 47 and 7% more BW than their Zn counterparts, respectively.

Hatfield et al. (1992, 1995) concluded that diets containing levels of Zn above NRC (1985) recommendations increased feedlot lamb performance and had a positive influence on ewe milk production and, ultimately, lamb weaning weight. However, Pond (1983) reported that 19 to 26 ppm Zn was adequate for normal weight gain and feed utilization in growing lambs.

**Ewe Cu Status**

Ewes supplemented with ZnCu had higher liver and fecal Cu concentrations than Zn-supplemented ewes. Similarly, Apgar and Kornegay (1996) reported an increase in fecal Cu in Cu-supplemented pigs compared with control pigs. Although Wellington et al. (1998)
Table 3. Effects of form of supplemental Zn with or without Cu on concentrations of Cu and Zn in feces at the end of the studyab

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Complex</th>
<th>Sulfate</th>
<th>P-values for contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal Cu, ppm</td>
<td>21.6</td>
<td>18.3</td>
<td>39.6</td>
<td>Control vs Zn vs Complex</td>
</tr>
<tr>
<td>Fecal Zn, ppm</td>
<td>49.9</td>
<td>237.8</td>
<td>233.3</td>
<td>Control vs Zn vs Complex</td>
</tr>
</tbody>
</table>

*No supplement type (Zn and ZnCu) \(\times\) form (complex and sulfate) interaction \((P > 0.35)\).
*Supplements were formulated to provide 90 and 10 mg/kg DM daily, respectively, of Zn and Cu.
*Control = no supplemental Zn or Cu.
*Complex = Availa-Zn 100 (complexed metal amino acid with 10% Zn and 20% total amino acids) and Availa-Cu 100 (complexed metal amino acid with 10% Cu and 20.6% total amino acids).
*Sulfate = Zn sulfate (36.36% Zn) and Cu sulfate (25.54% Cu).

Table 4. Effects of form of supplemental Zn with or without Cu on concentrations of Cu and Zn in the liver (DM basis) and serum alkaline phosphatase activity (SAPA) averaged over d 14 to 56 of supplementationab

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Complex</th>
<th>Sulfate</th>
<th>P-values for contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver Cu, ppm</td>
<td>605.7</td>
<td>564.9</td>
<td>644.0</td>
<td>Control vs Zn vs Complex</td>
</tr>
<tr>
<td>Liver Zn, ppm</td>
<td>121.5</td>
<td>139.0</td>
<td>145.6</td>
<td>Control vs Zn vs Complex</td>
</tr>
<tr>
<td>SAPA, sigma unit/mL</td>
<td>1.36</td>
<td>1.23</td>
<td>1.19</td>
<td>Control vs Zn vs Complex</td>
</tr>
</tbody>
</table>

*No supplement type (Zn and ZnCu) \(\times\) form (complex and sulfate) interaction \((P > 0.35)\). No effect of time or time \(\times\) treatment interactions \((P > 0.10)\) except for a time \(\times\) Zn vs ZnCu interaction \((P = 0.08)\) for Liver Cu, which is presented in Figure 1.
*Supplements were formulated to provide 90 and 10 mg/kg DM daily, respectively, of Zn and Cu.
*Control = no supplemental Zn or Cu.
*Complex = Availa-Zn 100 (complexed metal amino acid with 10% Zn and 20% total amino acids) and Availa-Cu 100 (complexed metal amino acid with 10% Cu and 20.6% total amino acids).
*Sulfate = Zn sulfate (36.36% Zn) and Cu sulfate (25.54% Cu).
*Liver Cu concentration on d 0 as a covariable.
*Liver Zn concentration on d 0 as a covariable.

**Eckert et al. (1999) reported that liver Cu concentrations tended to decrease when higher amounts of Cu were fed from proteinate but increased when greater amount of Cu were fed from sulfate. However, when low levels of Cu were fed, ewes fed proteinate tended to have higher liver Cu concentrations than those fed the sulfate form of Cu. These authors concluded that Cu proteinate maintained higher liver Cu when fed at normal levels and, when fed in excess amounts, will result in less liver Cu accumulation than Cu from sulfate. Liver Cu concentrations in our study ranged from 525 to 625 ppm. All of these values are within the “high” category reported by Puls (1994). However, it is not
clear whether the values reported by Puls (1994) are based on live animals or are values noted postmortem.

Fecal Cu concentration did not differ between complex- and sulfate-supplemented ewes. Nockels et al. (1993) also reported no difference between organic and inorganic forms of Cu on fecal Cu output.

**Ewe Zn Status**

Ewes supplemented with complex Zn and Cu had both higher liver and fecal Zn concentrations and a tendency for lower serum alkaline phosphatase than did ewes supplemented with sulfate forms of Zn and Cu. Nockels et al. (1993) reported that, after 30 d of feeding either Zn sulfate or Zn methionine to steers, no difference in fecal Zn, urinary excretion, apparent absorption, or retained Zn were noted. Spears (1989) stated that the advantage of organic Zn compared with inorganic Zn was higher retention rather than improved absorption. Although we did not measure Zn absorption, differences in liver and fecal Zn concentrations in our study may indicate that complex forms of Zn and Cu are metabolized differently than sulfate forms.

Other studies with sheep, cattle, and pigs have reported mixed results on the influence of form of Zn supplement on liver Zn concentrations. Engle et al. (1997) reported no influence of Zn source (sulfate, lysine, or methionine) on liver Zn concentrations during a Zn repletion phase in marginally Zn-deficient calves. Schell and Kornegay (1996) found no influence of form of supplemental Zn (oxide, methionine, lysine, and sulfate) on liver Zn concentrations in pigs fed 1000 mg/kg of Zn. However, at 2,000 and 3,000 mg of supplemental Zn/kg, liver Zn concentration was higher in Zn sulfate than in organic forms of supplemental Zn. Lambs supplemented with 360 mg Zn/kg (10 times NRC [1985] recommendations) in the form of Zn oxide, sulfate, methionine, and lysine did not differ in mean bone, bone marrow, cornea, skin, hoof, or muscle Zn concentration (Rojas et al., 1995). Although liver Zn did not differ between lambs supplemented with Zn sulfate and Zn methionine, Zn lysine-supplemented lambs had a higher liver Zn concentration than either Zn sulfate- or Zn methionine-supplemented lambs.

Rojas et al. (1996) concluded that at adequate levels of dietary Zn, bioavailability of supplemental Zn sources might be less important than under conditions of limited dietary Zn. In our study, forage Zn was approximately one-third the NRC (1985) recommended levels. This may explain, in part, the differences in results presented by Rojas et al. (1996) and our study. Liver Zn concentrations in our study range from 115 to 155 ppm, which are within the “adequate” range reported by Puls (1994).

Blood alkaline phosphatase activity has been used as an indication of animal Zn status. Wan et al. (1993) and Kraus et al. (1997) reported higher plasma alkaline phosphatase activity in Zn-adequate than Zn-deficient rats. Healy and Davis (1975) reported that total serum alkaline phosphatase activity increased more in lambs fed 100% wheat or 67% wheat and 33% alfalfa than in lambs fed diets containing 33% wheat and 67% alfalfa or diets composed of 100% alfalfa hay. These results reflect the Zn concentrations of wheat and alfalfa. In dogs, alkaline phosphatase did not respond to level or form of Zn supplementation in a consistent manner (Lowe and Wiseman, 1998). In our study, serum alkaline phosphatase activity did not differ between supplemented and control ewes. It is not clear why serum alkaline phosphatase activity tended to be greater in sulfate- than in complex-supplemented ewes; possibly these results indicate that complex forms of minerals are metabolized differently than sulfate forms. In pigs, serum alkaline phosphatase activity was not affected by source of Zn either as Zn sulfate or as chelated Zn (Swinkels et al., 1996).

Liver Zn concentrations were higher in Cu proteinate- than Cu sulfate-supplemented rats (Du et al., 1996). In addition, these authors reported that liver Zn concentrations were higher in the low (5 ppm) than the high (15 ppm) supplemental Cu groups. In contrast, Apgar and Kornegay (1996) and Eckert et al. (1999) reported that source and level of Cu supplement had no influence on liver Zn levels. In our study, ZnCu-supplemented ewes tended to have higher liver Zn concentration and lower fecal Zn concentration than did Zn-supplemented ewes. Possibly, feeding Zn in the presence of Cu increases both absorption and retention.

**Implications**

Complex forms of Zn and ZnCu increase liver Zn and Cu concentrations compared with sulfate forms of Zn and ZnCu. However, feeding high concentrations of Zn (100 mg/kg for 56 d) did not negatively impact liver Cu status. These results indicate that sheep producers wishing to feed high levels of supplemental Zn may do so without risk of adversely affecting the animal’s copper status.

**Literature Cited**


