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# Relative bioavailability of organic bis-glycinate bound copper relative to inorganic copper sulfate in beef steers fed a high antagonist growing diet

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## Abstract

To assess the relative bioavailability of bis-glycinate bound Cu, 90 Angus-cross steers (265 ± 21 kg) were blocked by body weight (**BW**) to pens with GrowSafe bunks and randomly assigned to dietary treatments (14 to 18 steers/treatment): 0 mg supplemental Cu/kg dry matter (**DM**; **CON**), 5 or 10 mg supplemental Cu/kg DM as Cu sulfate (**CS5**; **CS10**) or bis-glycinate bound Cu (**GLY5**; **GLY10**). Steers received a high antagonist growing diet (analyzed 4.9 mg Cu/kg DM, 0.48% S, and 5.3 mg Mo/kg DM). Steers were weighed at the beginning (days 1 and 0) and end (days 125 and 126) of the trial to determine average daily gain (**ADG**) and gain:feed (**G:F**). Blood was collected from all steers on days 0, 28, 56, 84, and 126. Liver samples were collected on days –3 or –2 and day 123 or 124. Data were analyzed using ProcMixed of SAS (experimental unit = steer; fixed effect = treatment and block). Plasma Cu was analyzed as repeated measures (repeated effect = day). Plasma and liver Cu concentrations were regressed against total Cu intake using ProcGLM to calculate relative bioavailability of GLY. Final BW and overall ADG were greatest for CS5 and CS10 and least for CON and GLY5 ($P = 0.01$). Overall, DMI was not affected by treatment ($P = 0.14$), but overall G:F tended to be greatest for CS5, CS10, and GLY5 and least for CON ($P = 0.08$). Total and supplemental Cu intake was greatest for steers supplemented either source at 10 mg Cu/kg DM and least for CON ($P < 0.01$). However, total and supplemental Cu intake was greater for CS5 than GLY5 ($P < 0.01$). Final liver Cu concentrations were greatest for CS10, least for CON, CS5, and CS10, and intermediate for GLY10 ($P < 0.01$). Final plasma Cu was greatest for steers supplemented either source at 10 mg Cu/kg DM ($P < 0.01$). Relative bioavailability of GLY was 82% compared to CS based on liver Cu ($P < 0.01$) but did not differ based on plasma Cu ($P = 0.60$). The lesser bioavailability of GLY relative to CS could be due to a high concentration of dietary antagonists and lower solubility of GLY (68.9% relative to CS) in pH conditions (5.2) similar to the ruminal pH of a beef animal consuming a high concentrate diet. Future studies should examine the effects of bis-glycinate bound Cu fed in blended combination with inorganic Cu sulfate to determine the most effective blend of sources for feedlot cattle experiencing varying amounts of dietary Cu antagonists.

**Key words:** amino acid chelate, cattle, solubility, thiomolymdate, trace mineral

**Abbreviations**

| | |
|---|---|
| ADG | average daily gain |
| BW | body weight |
| CS | copper sulfate |
| DM | dry matter |
| DMI | dry matter intake |
| G:F | gain:feed |
| GLY | bis-glycinate bound Cu |
| ICP-OES | inductively coupled plasma optical emissions spectrometry |
| TMR | total mixed ration |

## Introduction

Copper has many vital roles in growth, reproduction, and bone development through its involvement in enzyme cofactors and reactive proteins (Suttle, 2010). Copper concentrations of corn-based diets are typically well below the requirements of growing cattle (2 vs. 10 mg Cu/kg DM; NASEM, 2016; Niedermayer et al., 2018) and the bioavailability of dietary Cu is variable due to the presence of dietary antagonists like neutral detergent fiber, crude protein, S, and Mo (Ivan and Viera, 1981; Kabaija and Smith, 1988; Suttle, 2010). For example, under pH conditions found in the rumen, S and Mo react to form thiomolybdates, which can bind Cu in the gastrointestinal tract and inhibit absorption or enter circulation and peripheral tissues where they inhibit Cu dependent enzyme activity (Gould and Kendall, 2011). Therefore, feedlot cattle are typically provided supplemental Cu to prevent deficiency.

Traditional supplementation of Cu has been in the form of inorganic Cu sulfate ($CuSO_4$), which disassociates in the rumen, leaving Cu to form insoluble complexes with thiomolybdates (Suttle, 1991). Supplementation of organic trace mineral sources, such as amino acid chelates, has increased in popularity among feedlot nutritionists (Samuelson et al., 2016). Chelation prevents mineral solubility and unfavorable interactions in the gastrointestinal tract, theoretically improving intestinal absorption (Ashemead, 1993). While amino acid chelates have indeed been shown to improve Cu bioavailability relative to $CuSO_4$ (Hansen et al., 2008), others have reported no difference in bioavailability between inorganic and organic Cu sources (VanValin et al., 2019). The current study's objective was to calculate the bioavailability of an organic Cu source (bis-glycinate bound Cu; **GLY**) relative to inorganic $CuSO_4$ (**CS**). Relative bioavailability of Cu sources is best assessed under conditions of low Cu status and requires 2 concentrations of supplemental Cu. Therefore, steers were fed a high antagonist diet to deplete Cu status and received either 5 or 10 mg supplemental Cu/kg DM. Additionally, this study sought to determine the effects of supplemental Cu source and concentration on feedlot performance and Cu status of beef steers. It was hypothesized bis-glycinate bound Cu would have superior bioavailability relative to $CuSO_4$ because of decreased susceptibility to ruminal antagonists and increased intestinal absorption.

## Materials and Methods

All experimental procedures were approved by the Iowa State University Institutional Animal Care and Use Committee (log number 19 to 175).

### Animals and experimental design

Ninety Angus steers (265 ± 21 kg) were randomly allocated to partially covered concrete pens (16 pens; 4 m × 13.3 m) at the Iowa State University Beef Nutrition Research Unit (Ames, IA). Each pen contained a separate GrowSafe feed bunk (GrowSafe System, Calgary AB, Canada) to allow for measurement of individual feed intake and an automatic water tank was shared between adjacent pens. Upon arrival, cattle were fed a common receiving diet and acclimated to GrowSafe bunks for 15 d. Prior to trial initiation (day −10), steers received individual visual and electronic identification tags were vaccinated (Bovi-Shield Gold 5, Zoetis Animal Health, Parsippany, NJ; Vision 7 with SPUR, Merck Animal Health, Madison, NJ; Pinkeye Shield XT4, Elanco Animal Health, Greenfield IN), and dewormed (Dextomax Injectable Solution, Zoetis Animal Health). All steers received an anabolic implant (Component TE-IS with Tylan, Elanco Animal Health) at trial initiation (day 0). Based on initial processing body weight (**BW**), steers were randomly allocated to form 3 weight blocks. Pens within block were then randomly assigned to 1 of 5 dietary treatments ($n$ = 14 to 18 steers/treatment): no supplemental Cu (**CON**), 5 mg supplemental Cu/kg dry matter (**DM**) as $CuSO_4$ (**CS5**), 10 mg supplemental Cu/kg DM as $CuSO_4$ (**CS10**), 5 mg supplemental Cu/kg DM as bis-glycinate bound Cu (**GLY5**), or 10 mg supplemental Cu/kg DM as bis-glycinate bound Cu (**GLY10**). Cattle were fed a common corn silage-based diet daily at 0730 hours (Table 1; analyzed 4.9 mg Cu/kg DM) and had ad libitum access to feed. Dietary treatments were incorporated into the total mixed ration (**TMR**) as supplemental Cu premixes using dried distillers grains as a carrier. Antagonists (0.11% S as Ca sulfate and 5 mg Mo/kg DM as Na molybdate) were supplemented to all steers to aid in depletion of Cu status. Average daily gain (**ADG**) and feed efficiency (gain:feed; **G:F**) were calculated using the average of 2 consecutive days BW collected prior to feeding at the beginning (days −1 and 0) and end (days 125 and 126) of the trial.

**Table 1.** Ingredient and nutrient composition of common diet fed to steers

| DM, % as fed basis | 55 |
|---|---|
| Ingredient, % DM basis | |
| Corn silage | 40 |
| Sweet bran[1] | 40 |
| Dried distillers grains | 15 |
| Microingredients[2] | 5 |
| Supplemental Cu premix[3] | - |
| Analyzed composition[4] | |
| Neutral detergent fiber, % | 29.9 |
| Crude protein, % | 19.3 |
| Ether extract, % | 4.5 |
| S, % | 0.48 |
| Cu, mg/kg diet DM | 4.9 |
| Mo, mg/kg diet DM | 5.3 |

[1]Branded wet corn gluten feed (Cargill, Wayzata, MN).
[2]Mineral supplement provided per kg of total diet: 0.15 mg/kg Co (cobalt carbonate hydrate), 20 mg/kg Mn (manganese sulfate monohydrate), 0.1 mg/kg Se (sodium selenite), 30 mg/kg Zn (zinc sulfate), 5 mg/kg Mo (sodium molybdate dihydrate) and 0.5 mg/kg I (calcium iodate); remaining components (included as % of diet DM): dried distillers grains 2.77%, limestone 1.18%, vitamin A and E premix, 0.1%, $CaSO_4$ 0.6%, salt 0.31% and Rumensin 90 (Elanco Animal Health, Greenfield, IN) 0.0135%.
[3]Supplemental Cu premixes were composed of dried distillers grains and provided either 5 or 10 mg of supplemental Cu/kg DM from $CuSO_4$ or bis-glycinate bound Cu.
[4]All analyses, except Cu, were conducted by Dairyland Laboratories, Inc. (Arcadia, WI).

### Sample collection and analytical procedures

Total mixed ration samples for each respective treatment were collected weekly and frozen at –20 °C, then dried at 70 °C for 48 hr to determine diet DM. Weekly TMR DM values for each respective treatment were applied to steer as-fed intakes downloaded from the GrowSafe system to determine individual steer dry matter intake (**DMI**). Dried TMR samples were ground (Retsch ZM 100; Retsch GmbH, Haan, Germany) through a 2 mm screen, composited within treatment, and sent to a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI) for analysis of neutral detergent fiber, crude protein, ether extract, S, and Mo (AOAC 1999a; AOAC 1999b; AOAC 2005).

Blood samples were collected before the daily feeding (~0800 hr) from all steers on days 0, 28, 56, 84, and 126 via jugular venipuncture into two 6 mL trace element $K_2$EDTA blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ). Tubes were stored on ice until arrival at the laboratory and centrifugation at 1,000 × *g* for 20 min at 4 °C (Sorvall Legend X1F; Thermo Fisher Scientific Inc., Waltham, MA). Plasma was then aliquoted into microcentrifuge tubes and stored at –20 °C until preparation for trace mineral analysis. Liver biopsies were conducted on all steers at least 2 hr postfeeding using a method previously described (Engle and Spears, 2000). To facilitate sample collection, liver biopsies were performed across consecutive days near trial initiation (days –3 or –2) and trial completion (days 123 or 124) with treatments and blocks balanced across sampling days. Pens were brought to the working facility in the same order for each biopsy timepoint to ensure similar length between biopsies. Liver samples were stored on ice during collection, then stored at –20 °C until drying at 70 °C in a forced-air oven for ~5 d.

Dried liver and TMR (composited within treatment) samples were digested in a microwave (CEMS MARSXpress; Matthews, NC) using trace mineral grade nitric acid and then diluted to 20% nitric acid for trace mineral analysis as previously described (Richter et al., 2012; Pogge and Hansen, 2013). Plasma samples were prepared for trace mineral analysis as previously described by Pogge and Hansen (2013). Liver, TMR, and plasma samples were analyzed for trace mineral concentrations via inductively coupled plasma optical emissions spectrometry (**ICP-OES**; Optima 7000 DV; Perkin Elmer; Waltham, MA). A bovine liver standard (product #1577c; National Institute of Standards and Technology; Gaithersburg, MD) or serum standard (product #66816; UTAK Laboratories Inc., Valencia, CA) was included on each run to verify instrument accuracy. Yttrium (product #N9300167; Perkin Elmer) was added (1 µL Y/1 mL sample) to all samples as an internal standard to account for normal sample flow introduction fluctuation. Copper concentrations of TMR composites were multiplied by steer DMI to calculate total Cu intake for the 126-d feeding period. Supplemental Cu intake was calculated by subtracting the Cu concentration of the CON TMR from the Cu concentration of each respective treatment TMR; this value was then multiplied by steer DMI for the 126-d trial.

### Solubility of Cu sources

Solubility of CS and GLY was determined based on methods described by Spears et al. (2004). First, 0.05 g of each Cu source was weighed, in duplicate, and placed in an acid washed Erlenmeyer flask. Next, 100 mL of solvent (deionized water, pH = 5.2 or 0.1% hydrochloric acid, pH = 2.1) was added to the flask. Flasks were then covered in parafilm and placed in a shaking incubator set at 39 °C. Hydrochloric acid flasks were incubated for 1 hr while deionized water flasks were incubated for 24 hr. Following incubation, samples were filtered (Whatman Grade 541, hardened ashless filter paper; Whatman plc, Maidstone, UK), mixed, and stored in 50 mL conical tubes. The filtrate was diluted 1:10 in 10% nitric acid prior to analysis of Cu concentrations via ICP-OES. Solubility of CS was set to 100% and solubility of GLY was calculated relative to CS.

### Statistical analysis

This study was conducted as a generalized randomized block design with steer as the experimental unit. Four steers (1 CON, 2 CS10, and 1 GLY10) were removed from the trial due to injuries. Data were analyzed using the Mixed Procedure of SAS (SAS 9.4, Cary, NC). The model included the fixed effects of treatment and block. Initial BW was used as a covariate in analysis of other performance data to improve model fit based on Akaike Information Criterion. Liver Cu concentrations were $\log_{10}$ transformed to fit assumptions of normality and back transformed LSM and SEM are presented; initial liver Cu concentrations were used as a covariate in analysis of final liver Cu concentrations. Plasma Cu concentrations were analyzed as repeated measures with the fixed effects of treatment, day, and treatment × day with initial plasma Cu concentrations used as a covariate. Final liver and plasma Cu concentrations were regressed against total Cu intake using Proc GLM of SAS to determine slope coefficients as described by Littell et al. (1998). Relative bioavailability was calculated by dividing the slope of the test Cu source (GLY) by the slope of the standard Cu source (CS). Initial liver and plasma Cu concentrations served as covariates in analysis of relative bioavailability. Outliers were assessed using Cook's D statistic and studentized residuals; one GLY5 steer was identified as an outlier for G:F and removed from all performance analyses. Four steers (1 CS5, 2 CS10, and 1 GLY10) were identified as outliers for DMI or had no final liver biopsy and were removed from all data analyses. Significance was defined as $P \leq 0.05$ and tendencies were defined as $0.05 < P \leq 0.10$.

## Results

### Diet analysis and solubility of Cu sources

The common diet fed to all steers analyzed 0.48% S and 5.3 mg Mo/kg DM (Table 1). Analyzed Cu concentrations of TMR samples for each treatment were 4.9, 9.7, 14.2, 9.4, and 13.7 mg Cu/kg DM for CON, CS5, CS10, GLY5, and GLY10, respectively. Thus, supplemental Cu was provided at 0, 4.9, 9.3, 4.5, and 8.9 mg Cu/kg DM for CON, CS5, CS10, GLY5, and GLY10, respectively. Relative to CS, GLY was 68.9% soluble in deionized water (pH = 5.2; $P = 0.10$; Table 2) and 80.6% soluble in hydrochloric acid (pH = 2.1; $P = 0.32$).

### Growth performance

Initial BW did not differ due to treatment ($P = 0.65$; Table 3). Steers supplemented CS at 5 or 10 mg/kg DM had the greatest final BW and greatest overall ADG ($P = 0.01$). Final BW and overall ADG were least for CON and GLY5 while GLY10 did not differ from any other treatment ($P = 0.01$). Overall, DMI was not affected by dietary treatments ($P = 0.14$). Overall G:F tended to be greatest for CS5, CS10, and GLY5 ($P = 0.08$). Control steers tended to exhibit the lowest overall G:F while GLY10 tended not to differ from any other treatment ($P = 0.08$).

### Liver and Plasma Cu and Relative Bioavailability

Effects of dietary Cu treatment on liver and plasma Cu concentrations as well as total and supplemental Cu intake are shown in Table 4. By experimental design, total and

supplemental Cu intake was least for CON and greatest for steers supplemented either source at 10 mg Cu/kg DM ($P < 0.01$). However, steers supplemented 5 mg Cu/kg DM from CS consumed more total and supplemental Cu than those supplemented 5 mg Cu/kg DM from GLY ($P < 0.01$). There was no difference in initial liver Cu concentrations across treatments ($P = 0.92$). After 124 d, final liver Cu concentrations were greatest for CS10, least for CON, CS5, and GLY5, and intermediate for GLY10 ($P < 0.01$). Regardless of treatment, plasma Cu concentrations numerically decreased from d 0 (1.05 mg/L) to d 28 (0.75 mg/L). Plasma Cu concentrations were similar among treatments on days 28, 56, and 84; however, final (day 126) plasma Cu concentrations were greater for steers supplemented either source at 10 mg Cu/kg DM than those supplemented 0 or 5 mg Cu/kg DM (treatment × day $P < 0.01$; Figure 1). From days 28 to 56, plasma Cu concentrations decreased for CON, GLY5, and GLY10 but remained constant for CS5 and CS10. From days 56 to 84, plasma Cu concentrations increased for GLY5 and GLY10 but remained constant for CON, CS5, and CS10. From days 84 to 126, plasma Cu concentrations decreased for CON and GLY5, remained constant for CS5, and increased for CS10 and GLY10. Based on liver Cu, calculated relative bioavailability of GLY was 82% relative to CS ($P < 0.01$; Table 5), driven by differences in final liver Cu concentrations among sources when supplemented at 10 mg/kg DM. However, final plasma Cu concentrations were similar among sources when supplemented at 5 or 10 mg/kg DM (Table 4).

**Table 2.** Solubility of Cu sulfate (CS) and bis-glycinate bound Cu (GLY)

| | | | Solubility, % | | | |
|---|---|---|---|---|---|---|
| Diluent | pH | Incubation time, h | CS[1] | GLY | SEM | P-value |
| Deionized water | 5.2 | 24 | 100.0 | 68.9 | 4.10 | 0.10 |
| Hydrochloric acid | 2.1 | 1 | 100.0 | 80.6 | 8.74 | 0.32 |

[1]Solubility of CS was set to 100% and solubility of GLY was calculated relative to CS.

## Discussion

Ruminants linearly accumulate Cu in the liver as absorbed dietary Cu increases. Thus, liver Cu concentrations regressed against Cu intake can provide a useful estimate of bioavailability of 1 source of Cu vs. another. Steers had adequate Cu status at the beginning of the trial based on liver concentrations (Suttle, 2010), and liver Cu stores were successfully depleted over the 126 d trial by feeding a high antagonist diet (analyzed 0.48% S and 5.3 mg Mo/kg DM). While final liver Cu concentrations were similar between sources for steers fed 5 mg Cu/kg DM, differences emerge when comparing the sources fed at 10 mg Cu/kg DM and thus GLY appears to be less bioavailable than CS. As discussed earlier, amino acid metal chelates are often lowly soluble in the rumen, resulting in limited Cu availability. Indeed, Cu solubility results from the present study indicated GLY to be less soluble than $CuSO_4$ at pH 5.2, which is slightly lesser than normal ruminal pH in beef cattle (5.6; Nagaraja and Titgemeyer, 2007). This is important in the context of high antagonist diets, as the thiomolybdate complex (primarily di [$MoS_2O_2$], tri [$MoS_3O$], and tetra [$MoS_4$]-thiomolybdates) caused by the sulfide and molybdate interaction occurs in the reducing environment of the rumen (Clarke and Laurie, 1980; El-Gallad et al., 1983). Thiomolybdates bind to soluble Cu, preventing it from being absorbed; however, if insufficient ruminally soluble Cu is available, thiomolybdates are known to be absorbed across the rumen wall (Gould and Kendall, 2011). Absorbed thiomolybdates can then enter the liver via portal circulation and form complexes with Cu containing proteins like metallothionein; these complexes may be excreted from the liver and the body via biliary excretion (Suttle, 2012), thus depleting Cu stores in the animal.

Similar to results of the current study, steers supplemented $CuSO_4$ for 84 d in conjunction with a high antagonist diet (0.25% S and 6.8 mg/kg Mo) had greater final liver Cu than steers supplemented Cu lysine (VanValin et al., 2019). This may mean that feeding supplemental Cu strictly from an organic source that is not as ruminally soluble as inorganic $CuSO_4$ is not ideal when high amounts of ruminal antagonists are present. Nevertheless, Hansen et al. (2008) reported Cu glycinate to be more bioavailable than $CuSO_4$ in steers fed diets containing high amounts of antagonists (0.39% S; 2 mg Mo/kg DM for the first 120 d and 6 mg Mo/kg DM for the last 28 d). Contrasting results

**Table 3.** Effect of supplemental Cu source and concentration on feedlot performance of steers fed a high antagonist growing diet

| | Treatment[1] | | | | | | Treatment |
|---|---|---|---|---|---|---|---|
| | CON | CS5 | CS10 | GLY5 | GLY10 | SEM[2] | P-value |
| *n*, steers | 17 | 17 | 14 | 17 | 16 | | |
| Initial BW[3,4], kg | 281 | 277 | 283 | 281 | 285 | 3.6 | 0.65 |
| Final BW[3], kg | 503[b] | 526[a] | 523[a] | 507[b] | 516[a,b] | 5.7 | 0.01 |
| Overall performance[5] | | | | | | | |
| ADG, kg | 1.77[b] | 1.95[a] | 1.92[a] | 1.80[b] | 1.87[a,b] | 0.045 | 0.01 |
| DMI, kg | 10.3 | 10.4 | 10.4 | 9.7 | 10.3 | 0.25 | 0.14 |
| G:F | 0.172[y] | 0.188[x] | 0.186[x] | 0.188[x] | 0.182[x,y] | 0.005 | 0.08 |

[1]Treatments = 0 mg supplemental Cu/kg DM (CON), 5 or 10 mg supplemental Cu/kg DM as inorganic Cu sulfate (CS5; CS10) or chelated bis-glycinate bound Cu (GLY5; GLY10).
[2]Highest SEM of any treatment reported.
[3]Initial BW = average of consecutive day BW collected on days −1 and 0; final BW = average of consecutive day BW collected on days 125 and 126.
[4]Initial BW used as a covariate in analysis of other performance variables.
[5]Feedlot performance for the overall trial (days 0 to 126); ADG = average daily gain; DMI = dry matter intake; G:F = gain:feed.
[a,b]Means with unlike superscripts indicate a difference ($P \leq 0.05$) between treatments.
[x,y]Means with unlike superscripts indicate a tendency for a difference ($0.05 < P \leq 0.10$) between treatments.

**Table 4.** Effect of supplemental Cu source and concentration on liver and plasma Cu concentrations and Cu intake of steers fed a high antagonist growing diet

| | Treatment[1] | | | | | | Treatment |
|---|---|---|---|---|---|---|---|
| | CON | CS5 | CS10 | GLY5 | GLY10 | SEM[2] | *P*-value |
| Liver Cu[3], mg/kg DM | | | | | | | |
| Initial | 175.5 | 174.9 | 176.0 | 177.6 | 189.5 | 5.68 | 0.91 |
| Final | 7.4[c] | 9.7[c] | 51.0[a] | 8.9[c] | 19.3[b] | 2.78 | <0.01 |
| Plasma Cu[4], mg/L | | | | | | | |
| Initial | 1.04 | 1.01 | 1.04 | 1.12 | 1.02 | 0.037 | 0.15 |
| Final | 0.56[b] | 0.63[b] | 0.90[a] | 0.61[b] | 0.85[a] | 0.050 | <0.01 |
| Cu intake[5], g | | | | | | | |
| Total | 6.5[d] | 12.9[b] | 18.8[a] | 11.5[c] | 18.3[a] | 0.37 | <0.01 |
| Supplemental | 0.0[d] | 6.4[b] | 12.3[a] | 5.5[c] | 11.8[a] | 0.21 | <0.01 |

[1]Treatments = 0 mg supplemental Cu/kg DM (CON), 5 or 10 mg supplemental Cu/kg DM as inorganic Cu sulfate (CS5; CS10) or chelated bis-glycinate bound Cu (GLY5; GLY10).
[2]Highest SEM of any treatment reported.
[3]Initial (days −3 or −2) liver Cu concentrations were utilized as a covariate in analysis of final (days 123 or 124) liver Cu concentrations; data were $\log_{10}$ transformed prior to analysis and are presented as back transformed means and SEM.
[4]Initial (day −0) plasma Cu concentrations were utilized as a covariate in analysis of final (day 126) plasma Cu concentrations.
[5]Total and supplemental Cu intake for the 126-d trial calculated by multiplying steer DMI by Cu concentrations of the total mixed ration.
[a–d]Means with unlike superscripts indicate a difference ($P \leq 0.05$) between treatments.

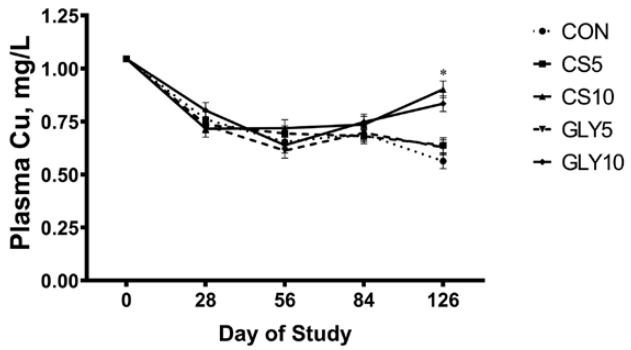


**Figure 1.** Effect of dietary Cu treatment and day of study (treatment × day $P < 0.01$) on plasma Cu concentrations of steers supplemented with 0 mg supplemental Cu/kg DM (**CON**), 5 or 10 mg supplemental Cu/kg DM as inorganic $CuSO_4$ (**CS5**; **CS10**), or chelated bis-glycinate bound Cu (**GLY5**; **GLY10**) in conjunction with a high antagonist diet for 126 d. Initial plasma Cu concentrations (day 0) were used as a covariate in analysis. *Treatment means were similar on days 28, 56, and 84; however, on day 126, plasma Cu concentrations were greater ($P \leq 0.05$) for CS10 and GLY10 relative to other treatments.

among studies could be due to differences in Cu concentrations of the basal diets. For example, the basal diet fed by Hansen et al. (2008) provided almost twice the amount of Cu (8.2 mg Cu/kg DM) compared to the basal diet fed in the current study (4.9 mg Cu/kg DM) and by VanValin et al. (2019; 4.5 mg Cu/kg DM). If this dietary Cu was ruminally soluble, the greater amount of basal Cu supplied by Hansen et al. (2008) may have prevented thiomolybdate entry into circulation and depletion of liver Cu in glycinate supplemented steers.

There was no difference in bioavailability between the 2 sources when using final plasma Cu concentrations. This

**Table 5.** Calculated relative bioavailability of Cu from sulfate (CS) and bis-glycinate (GLY) sources based on regression of liver or plasma Cu concentrations against total Cu intake by steers fed a high antagonist growing diet

| | | | | *P*-value |
|---|---|---|---|---|
| Cu measures[1] | Source | Slope ± SE | Relative bioavailability, % | CS vs. GLY |
| Liver | CS | 0.0683 ± 0.0086 | 100 | <0.01 |
| | GLY | 0.0563 ± 0.0090 | 82 | |
| Plasma | CS | 0.0310 ± 0.0068 | 100 | 0.60 |
| | GLY | 0.0294 ± 0.0070 | 95 | |

[1]Initial liver and plasma Cu used as covariates in each respective model.

suggests that steers fed GLY were able to maintain circulating Cu concentrations, which should provide adequate Cu for tissue demands, yet steers fed 10 mg Cu/kg DM from GLY apparently could not replenish liver Cu stores by day 124. It is unclear if this source difference would have resolved had the trial progressed further. It is also worth noting that the amount of Cu bound to thiomolybdates in plasma may have differed between sources. However, the current study utilized methods to assess total plasma Cu rather than TCA-insoluble Cu which can be used as an indirect measure of Cu bound to thiomolybdates in circulation (Gould and Kendall, 2011). At trial initiation, steers had plasma Cu concentrations in the adequate range (0.8 to 1.2 mg Cu/L; Underwood, 1977). Plasma Cu concentrations dropped substantially after 28 d of consuming the high antagonist diet. It was expected that plasma Cu would continue to drop over the 126-d feeding period. Instead, steers supplemented 10 mg Cu/kg DM from either source improved plasma Cu concentrations between days 84 and 126. This agrees with previous literature, which found 0 and 5 mg Cu/kg DM insufficient to maintain adequate plasma Cu concentrations in steers fed diets high in S and Mo (Hansen et al., 2008). Additionally, the increase in plasma Cu concentrations late in the feeding period for steers supplemented 10 mg Cu/kg DM suggests that the Cu requirement of finishing steers may be less than growing steers. Copper is known to play roles in enzymes needed for connective tissue cross-linking (Suttle, 2010) and formation of the extracellular matrix, which supports growth (Gentili and Cancedda, 2009). However, bone and muscle growth plateaus during the finishing phase, and fat deposition accumulates at an exponential rate. The difference in rate and composition of growth between growing and finishing cattle could influence biological demands for Cu and thus influence Cu requirements.

Previous research eludes to depression in intake as a result of high levels of Mo in the diet (5 mg/kg Mo; Kegley and Spears, 1994), and Cu supplementation has been reported to increase DMI of steers fed a high antagonist diet (2 to 6.9 mg Mo/kg DM; 0.30 to 0.39% S), regardless of Cu source (Spears et al., 2004; Hansen et al., 2008). In the current trial, overall DMI was not affected by supplemental Cu treatments. However, numerically lesser DMI for GLY5 (9.7 kg/d) relative to CS5 (10.4 kg/d) resulted in lesser total Cu intake for GLY5 (11.5 g) relative CS5 (12.9 g). These differences in Cu intake would have been accounted for in the bioavailability calculations as initial and final liver and plasma Cu concentrations were regressed against total Cu intake. The observed improvements in BW and ADG of

steers provided supplemental Cu at 5 or 10 mg/kg DM from CS compared with steers receiving no supplemental Cu agrees with past literature (Spears et al., 2004; Hansen et al., 2008). Interestingly, this performance advantage was not observed when steers were provided supplemental Cu at 5 mg/kg DM from GLY. VanValin et al. (2019) reported a tendency for steers supplemented inorganic Cu ($CuSO_4$) to have greater overall ADG compared with steers supplemented with organic Cu (Cu lysine). This may provide further evidence that providing supplemental Cu solely from organic sources is not ideal when high amounts of antagonists are present in the diet.

Relative to $CuSO_4$, bis-glycinate bound Cu was less bioavailable to growing steers fed a high antagonist diet based on liver Cu concentrations in the present study. According to a survey of consulting feedlot nutritionists, ~55% of respondents recommend a combination of inorganic and organic trace mineral sources for finishing cattle diets (Samuelson et al., 2016). Thus, future research needs to be conducted with blends (e.g., 25% organic/75% inorganic, etc.) to determine the optimal combination of Cu sources to support cattle growth. Because dietary Cu content and Cu antagonists vary widely across the world, it is important to assess bioavailability under conditions of low or moderate antagonistic pressure. Additionally, more research is needed to understand the solubility of bis-glycinate bound Cu and Cu requirements of finishing steers.

## Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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