Translational Animal Science, 2024, **8**, txae105
https://doi.org/10.1093/tas/txae105
Advance access publication 12 July 2024
Ruminant Nutrition

Determination of relative bioavailability of copper from copper glycinate in growing beef steers

Jacob A. Henderson, Emma K. Niedermayer-Conway, and Stephanie L. Hansen[1]

Department of Animal Science, College of Agriculture and Life Sciences, Iowa State University, Ames, IA 50011, USA
[1]Corresponding author: slhansen@iastate.edu

ABSTRACT

Chelated copper (**Cu**) sources, such as Cu glycinate (**CuGly**), may be more bioavailable relative to Cu sulfate ($\mathbf{CuSO_4}$) when fed to ruminants under antagonistic pressure. The objective of this study was to determine the bioavailability of CuGly (GemStone Cu; Phibro Animal Health) relative to $CuSO_4$ in steers fed a diet supplemented with 0.3% sulfur and 2 mg molybdenum/kg of dry matter (**DM**). Sixty Angus crossbred steers (n = 12 per treatment) averaging 288 ± 4.85 kg were enrolled in a 90-d study and fed a corn silage-based diet with one of five Cu supplementation strategies, including no supplemental Cu **(CON)**, 5 or 10 mg supplemental Cu from $CuSO_4$/kg DM, and 5 or 10 mg supplemental Cu from CuGly/kg DM. Steers were housed in pens equipped with GrowSafe feed bunks (GrowSafe Systems Ltd., Airdire, AB, Canada), with six steers per pen. Growth performance, liver Cu, and plasma Cu were analyzed in the MIXED procedure of SAS 9.4 (SAS Inst. Inc, Cary, NC) with orthogonal contrasts to compare CON vs. 5 mg Cu/kg DM, CON vs. 10 mg Cu/kg DM, 5 vs. 10 mg Cu/kg DM, and $CuSO_4$ vs. CuGly. Copper indices were regressed against Cu intake and slopes were calculated using the GLM procedure SAS. Dietary Cu supplementation did not affect steer body weights on days 0, 28, 56, or 90 ($P \geq 0.52$), average daily gain, dry matter intake, or gain:feed ($P \geq 0.36$). Final plasma Cu concentration did not differ between CON vs. 5 mg Cu/kg DM ($P = 0.79$), CON vs. 10 mg Cu/kg DM ($P = 0.65$), or 5 vs. 10 mg Cu/kg DM ($P = 0.39$). Steers receiving $CuSO_4$ tended to have greater final plasma Cu concentrations than those receiving CuGly ($P = 0.08$). Initial liver Cu concentration averaged 374 mg Cu/kg DM, which is considered highly adequate. No steers reached deficient Cu status by the end of the 90-d period. Control steers had lesser final liver Cu concentrations than supplemented steers ($P \leq 0.04$). Steers receiving 10 mg supplemental Cu/kg DM had greater liver Cu concentrations than those receiving 5 mg supplemental Cu/kg DM ($P = 0.01$). Copper source had no effect on final liver Cu concentrations ($P = 0.57$) and based on liver Cu and Cu intake the bioavailability of CuGly was similar to $CuSO_4$ (115%; $P = 0.27$). The initially high Cu status and the fact that cattle did not become Cu deficient may have impacted the relative bioavailability results, and more research is needed to investigate the role initial Cu status and antagonistic pressure play in the bioavailability of chelated Cu sources.

LAY SUMMARY

Beef producers have a variety of copper (**Cu**) supplements available, which may vary in susceptibility to antagonists. This 90-d study used 60 Angus-cross steers fed one of five Cu supplementation strategies to determine the bioavailability of Cu glycinate (**CuGly**) relative to the standard inorganic source, Cu sulfate ($\mathbf{CuSO_4}$). All diets included the Cu antagonists sulfur and molybdenum at 0.3% and 2 mg/kg DM, respectively. Total Cu intake for each steer was calculated, and liver and plasma Cu were determined on days 0 and 90. The calculated relative bioavailability of CuGly was 115% and was not different from $CuSO_4$. Several factors appear to affect Cu bioavailability assessments, including initial Cu status, availability of basal diet Cu, and the extent of Cu antagonistic pressure in addition to growth rates and other physiological demands of the animal.

Key words: beef cattle, bioavailability, copper glycinate, copper sulfate

INTRODUCTION

Copper (**Cu**) plays a vital role in the formation of various enzymes and cofactors necessary for proper metabolic function (Suttle, 2010). While Cu deficiency in beef cattle is prevalent in all parts of the world, it is especially challenging in areas where sulfur (**S**) and molybdenum (**Mo**) are present in the soil, water, and feedstuffs. In the rumen, S and Mo bind to form thiomolybdates, which have a high affinity for Cu (Price and Chesters, 1985). Once Cu is bound by thiomolybdates, it is no longer available for absorption at any point in the gastrointestinal tract (Suttle, 1974). Further, excess thiomolybdate can be absorbed through the rumen wall and into the blood and bind Cu, thus preventing the function of Cu-dependent enzymes (Gould and Kendall, 2011).

Inorganic Cu sulfate ($\mathbf{CuSO_4}$), is highly soluble in the rumen and can readily be bound by thiomolybdates, making it less available to the animal under antagonistic pressure. The reported bioavailability of chelated Cu sources is inconsistent. For example, Kincaid et al. (1986) found Cu proteinate to be more bioavailable than $CuSO_4$, while Wittenburg et al. (1990) found similar bioavailability between Cu proteinate and $CuSO_4$. Copper from Cu glycinate (**CuGly**) has been shown to solubilize in water without dissociating from the glycine amino acid (Phibro Animal Health Corporation, 2024). However, CuGly has been less extensively studied in ruminants; thus, the objective of this study was to determine the bioavailability of Cu from CuGly fed as GemStone Cu 240 (Phibro Animal Health Corporation, Teaneck, NJ) relative to Cu from $CuSO_4$ when fed in growing cattle diets containing high concentrations of S and Mo.

Received May 15, 2024 Accepted July 11, 2024.

MATERIALS AND METHODS

Animals and Experimental Design

All experimental procedures involving the use of animals in this experiment were reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee (log number 9-17-8608-B).

Sixty crossbred Angus steers (288 ± 26 kg) were used in a 90-d experiment conducted at the Iowa State University Beef Nutrition Farm (Ames, IA). Steers were blocked by body weight (**BW**) measured on days −6 and −7 into GrowSafe feed bunk-equipped pens (GrowSafe Systems Ltd., Airdire, AB, Canada; one bunk per pen) with six steers per pen. Steers were weighed and implanted (Component TE-IS, Elanco Animal Health, Indianapolis, IN) on day 0 and pens were randomly assigned to one of five dietary treatments: (1) **CON** (no supplemental Cu), (2) **Sul5** (5 mg supplemental Cu/kg DM from $CuSO_4$), (3) **Gly5** (5 mg supplemental Cu/kg DM from CuGly; GemStone Cu; Phibro Animal Health), (4) **Sul10** (10 mg supplemental Cu/kg DM from $CuSO_4$), and (5) **Gly10** (10 mg supplemental Cu/kg DM from CuGly). GrowSafe bunks recorded individual animal feed disappearance via radio frequency tags on each steer. All steers within a pen were assigned to the same treatment with two pens per treatment and 12 steers per treatment; steer was the experimental unit. Apart from Cu supplement, all diets were identical, corn silage-based diets and supplemented with 0.3% S from calcium sulfate and 2 mg Mo/kg DM from sodium molybdate; all other trace minerals were supplemented according to NASEM recommendations (2016). The composition of this common diet is displayed in Table 1. Cattle had ad libitum access to both feed and water. Feed was delivered once daily at approximately 0800 hours. Over the 90-d study, steers were weighed prior to feeding on days 0, 28, 56, 89, and 90 to determine growth performance. The average of the weights for each steer on days 89 and 90 were used to calculate the final BW. A 4% pencil shrink was applied to all live BW measurements.

Table 1. Composition of common diet

Ingredient	Percent of the diet (dry matter basis)
Corn silage	40
Corn	30
Dried distillers grains	20
Cu premix[a]	5
TM premix[b]	2.3
Limestone	0.5
Calcium sulfate	1.8
Salt	0.31
Vitamin A and E premix[c]	0.1
Rumensin 90	0.0135
Analyzed composition	
Crude protein, %	15.0
Neutral detergent fiber, %	25.0
Ether extract, %	6.1
Sulfur, %	0.56
Mo, mg/kg DM	2.9
Cu, mg/kg DM	6.0

[a]Respective Cu treatments were delivered as a premix with a dried distillers grains carrier.
[b]Trace mineral premix were delivered as a premix to provide: 0.15 mg Co/kg DM, 20 mg Mn/kg DM, 0.1 mg Se/kg DM, 30 mg Zn/kg DM, and 2 mg Mo/kg DM with a dried distillers grains carrier.
[c]Premix provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet DM.

Sampling and Analytical Procedures

Diet ingredient and total mixed ration (**TMR**) samples were collected weekly to determine dry matter (**DM**) content, which was used to calculate DM intake. After drying in a forced-air oven for 48 h (70 °C), feed samples were ground through a 2-mm screen (Retsch Zm100 grinder, Glen Mills, Inc., NJ). After grinding, monthly TMR samples were composited according to treatment.

Jugular blood samples were collected from all steers prior to feeding at the initiation of treatments (split across days −1 and −2) and at the end of the study (stratified by treatment across days 84 and 85) to determine initial and final plasma Cu concentration. Blood samples were collected into tubes containing potassium EDTA (Becton Dickenson, Rutherford, NJ), after which they were transported to the lab on ice. Samples were centrifuged at 1,000 × *g* for 10 min at 4 °C. Plasma was removed and stored at −20 °C until analyzed for Cu concentrations using inductively coupled plasma optical emission spectrometry (**ICP-OES**).

Liver biopsies were also conducted on all steers at least 2 h postsfeeding as described by Engle and Spears (2000), split across days −1 and −2 as well as stratified by treatment across days 84 and 85. Liver samples were then dried in a forced-air oven at 70 °C until completely dry (approximately 7 d).

After drying, feed and liver samples were acid digested (CMES MARSXpress, Matthews, NC) with trace mineral grade nitric acid prior to mineral analysis as described by Richter et al. (2012). Copper concentrations of feed, plasma, and liver samples were determined using ICP-OES. A bovine National Institute of Standards and Technology liver sample (US Department of Commerce, Gaithersburg, MD) was used to verify instrument accuracy on feed and liver runs while a UTAK (UTAK Laboratories, Valencia, CA) standard was used on plasma runs.

Statistical Analysis

Growth performance, liver, and plasma Cu data were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC), using block and treatment as fixed effects and pen as a random variable. The experimental unit for all data was steer (n = 12 steers per treatment). Initial liver and plasma Cu concentrations were used as a covariate when analyzing final liver and plasma Cu concentrations. Cook's D statistic was used to identify outliers with a cutoff value of 0.5; however, no outliers were detected. Single degree of freedom orthogonal contrasts were used to compare differences among treatment means. These comparisons included control vs. 5 mg Cu/kg DM, control vs. 10 mg Cu/kg DM, 5 mg Cu/kg DM vs. 10 mg Cu/kg DM, and sulfate vs. glycinate. The GLM procedure of SAS was used to determine the relative bioavailability of CuGly, using $CuSO_4$ as the standard source, by means of multiple linear regression and the slope-ratio method. Liver Cu was regressed on supplemental Cu intake for the 90-d period, using initial liver Cu concentration as a covariate. No transformations were performed, as data were found to be normal. Liver Cu data regression met the assumptions for the slope-ratio

assay based on Liu et al. (2012), so no adjustment factors were needed. Significance was $P \leq 0.05$, and tendencies were $0.05 < P \geq 0.1$. Data presented are LSMEANS and SEM.

RESULTS

Live Animal Performance

Live animal performance data are summarized in Table 2. There was no difference between treatments in steer BW on days 0, 28,56, or 90 ($P \geq 0.52$). Dietary Cu supplementation had no effect on days 0 to 90 ADG, DMI, or G:F ($P \geq 0.19$). Calculated supplemental Cu intakes, based on daily DMI, are displayed in Table 2 and were different between concentrations of Cu ($P < 0.01$) but not between sources ($P = 0.99$).

Plasma and Liver Cu Status

Plasma and liver Cu concentrations are presented in Table 3. Initial plasma and liver Cu concentrations collected before the start of the experiment were used as a covariate in the analysis of the respective tissue concentrations at the end of the 90-d period. There were no differences in final plasma Cu concentration between control vs. 5 mg supplemental Cu/kg DM ($P = 0.79$), control vs. 10 mg supplemental Cu/kg DM ($P = 0.65$), or 5 mg supplemental Cu/kg DM vs. 10 mg supplemental Cu/kg DM ($P = 0.39$). Steers receiving the $CuSO_4$ supplement tended to have greater final plasma Cu concentrations than those receiving the CuGly supplement ($P = 0.08$).

Initial liver Cu concentrations averaged 374 mg/kg DM and were similar among treatments. Control steers had lesser final liver Cu concentrations than steers that received 5 mg supplemental Cu/kg DM or 10 mg supplemental Cu/kg DM from either treatment ($P \leq 0.04$), and steers receiving 10 mg supplemental Cu/kg DM had greater liver Cu concentrations than those receiving 5 mg supplemental Cu/kg DM from either treatment ($P = 0.01$). Liver Cu concentrations were not different between sulfate and glycinate sources ($P = 0.57$).

Relative Bioavailability

Slopes and relative bioavailability results are presented in Table 4. The bioavailability of CuGly relative to $CuSO_4$ was estimated from liver Cu concentrations at the end of the 90-d supplementation period using multiple linear regression and the slope-ratio method. Compared with $CuSO_4$, the relative bioavailability of CuGly was 115.5% ($P = 0.27$).

DISCUSSION

Copper absorption and metabolism in beef cattle are heavily impacted by the presence of other minerals in feedstuffs or water. For example, Cu antagonism may become an issue when feeding distillers grains, which contain high concentrations of S due to the use of sulfuric acid in the production process (NASEM, 2016). Once Cu is bound to thiomolybdates in the rumen, it cannot be absorbed throughout the remainder of the digestive tract. If the source of Cu is not rumen soluble, it is unavailable for thiomolybdate binding in the rumen. Chelated Cu supplements, such as CuGly, are generally less soluble in the rumen than inorganic supplements, such as $CuSO_4$. Therefore, CuGly should facilitate improved Cu status in ruminants receiving high S or Mo diets compared to $CuSO_4$.

In the present study, CuGly had a numerically greater, but statistically insignificant, bioavailability of 115.5% compared to $CuSO_4$ after feeding 0.3% S and 2 mg Mo/kg for 90 d. At the start of the trial, liver Cu status averaged 374 mg/kg DM, which is considered highly adequate (Kincaid, 1999). As expected in the presence of antagonists, liver Cu concentrations decreased across all treatments. Control cattle experienced an 80% decrease in liver Cu, while steers receiving 5 mg supplemental Cu/kg DM, regardless of source, experienced a 65% decrease in liver Cu concentrations. Steers receiving 10 mg supplemental Cu/kg DM exhibited a 46% decrease in liver Cu concentrations. Despite this, no cattle reached deficient status during the 90-d experiment. Copper deficiency can result in the upregulation of Cu transporters, thus increasing the bioavailability of Cu (Liu et al., 2012). It is possible that Cu absorption rates in the present study were decreased due

Table 2. Effect of copper supplementation from copper sulfate or copper glycinate for 90 d on performance of growing beef steers

	Treatment[a,b]					SEM	Contrast *P*-values			
	Control	Sul 5	Gly 5	Sul 10	Gly 10		Control vs 5 mg/kg	Control vs 10 mg/kg	5 mg/kg vs 10 mg/kg	Sulfate vs Glycinate
day 0 BW, kg	289	284	289	288	289	4.8	0.67	0.94	0.66	0.59
day 28 BW, kg	348	345	347	345	345	4.8	0.69	0.57	0.83	0.88
day 56 BW, kg	387	390	390	392	390	5.5	0.68	0.57	0.84	0.88
day 90 BW, kg	440	445	446	444	443	6.5	0.69	0.52	0.75	0.97
days 0 to 90 ADG, kg/d	1.68	1.79	1.75	1.73	1.72	0.064	0.58	0.24	0.44	0.65
days 0 to 90 DMI, kg/d	9.56	9.39	9.94	9.80	9.53	0.267	0.75	0.76	0.99	0.61
days 0 to 90 G:F	0.177	0.192	0.177	0.177	0.180	0.0054	0.65	0.19	0.29	0.23
Daily supplemental Cu intake, mg/d	0	46.91	49.65	97.95	95.23	1.99	<0.01	<0.01	<0.01	0.99

[a]Treatments included: Control (no supplemental copper), Sul 5 (5 mg supplemental Cu/kg DM from $CuSO_4$), Gly 5 (5 mg supplemental Cu/kg DM from CuGly), Sul 10 (10 mg supplemental Cu/kg DM from $CuSO_4$), and Gly 10 (10 mg supplemental Cu/kg DM from CuGly).
[b]Dietary antagonists included 0.3% S from calcium sulfate and 2 mg Mo/kg DM from sodium molybdate.

Table 3. Effects of copper supplementation as copper sulfate or copper glycinate for 90 d on growing beef steer plasma copper and liver copper concentrations

	Treatment[a,b]					SEM	Contrast *P*-values			
	Control	Sul 5	Gly 5	Sul 10	Gly 10		Control vs. 5 mg/kg	Control vs. 10 mg/kg	5 mg/kg vs. 10 mg/kg	Sulfate vs. Glycinate
Plasma Cu, mg/L										
Initial	1.09	1.04	1.02	0.97	0.94	—	—	—	—	—
Final[c], day 84/85	0.78	0.79	0.75	0.84	0.75	0.034	0.79	0.65	0.39	0.08
Liver Cu, mg/kg DM										
Initial	392	350	364	391	372	—	—	—	—	—
Final[c], day 84/85	67	128	129	191	212	17.9	0.0384	0.0017	0.0092	0.5658

[a]Treatments included: Control (no supplemental copper), Sul 5 (5 mg supplemental Cu/kg DM from CuSO4), Gly 5 (5 mg supplemental Cu/kg DM from CuGly), Sul 10 (10 mg supplemental Cu/kg DM from CuSO4), and Gly 10 (10 mg supplemental Cu/kg DM from CuGly).
[b]Dietary antagonists included 0.3% sulfur from calcium sulfate and 2 mg Mo/kg DM from sodium molybdate.
[c]Initial plasma and liver copper concentrations were used as a covariate in analysis of final plasma and liver copper concentrations.

Table 4. Estimated relative bioavailability of copper glycinate compared to copper sulfate for 90 d on growing beef steers liver copper concentrations, based on multiple linear regression of liver copper on total supplemental copper intake[a]

Cu index[b,c]	Cu source	Slope ± SE	*P*-value[d]	Relative bioavailability, %
Liver Cu	Sulfate	13.92 ± 2.021	0.27	100
	Glycinate	16.08 ± 2.056		115.5

[a]Based on regression of Cu indices, liver Cu in mg/kg DM, on total supplemental Cu intake (g) of steers over the 90-d period.
[b]Regression based on final measurements following feeding a diet containing 0.3% sulfur from calcium sulfate and 2 mg Mo/kg DM from sodium molybdate for 90 d.
[c]Initial values were used as a covariate in analysis for final liver concentrations.
[d]*P*-value for slope between copper sources.

to the greater initial liver Cu concentrations and less time under antagonistic pressure to deplete liver Cu enough to cause increased absorption. These results suggest more research is needed to determine the impact initial Cu status has on overall absorption rates and bioavailability.

Results obtained across previous studies examining the bioavailability of chelated Cu sources have been widely variable. When comparing Cu lysine to $CuSO_4$ under varying concentrations of Mo and S antagonists, both Ward et al. (1993) and Kegley and Spears (1994) found similar bioavailability of Cu from Cu lysine and $CuSO_4$. However, Rabiansky et al. (1999) observed a greater bioavailability of Cu lysine in heifers that were initially Cu deficient, with fewer differences in relative bioavailability between $CuSO_4$ and Cu lysine in heifers that had greater initial liver Cu concentrations. These results in particular, similar to the present study, suggest greater initial liver Cu concentrations take longer to decrease, which in turn results in lesser observed relative bioavailability. Analysis of liver Cu concentrations of heifers from four distinct ranches purchased at a single sale barn in Nebraska revealed that heifers from one ranch had liver Cu concentrations well in excess of the 600 mg Cu/liver DM upper threshold for adequate liver Cu, while heifers from another ranch were well below the 125 mg Cu/liver DM lower threshold (Messersmith et al., 2021). These data demonstrate the wide variation in Cu status that animals from separate backgrounds may exhibit as they enter the feedlot. Given that bioavailability results vary depending on initial and final Cu status, more work is needed to determine how to best study and apply bioavailability findings to cowherds and feedlots, as cattle can vary widely in Cu status.

Hansen et al. (2008) found CuGly had a relative bioavailability of 131% compared to $CuSO_4$ after feeding 2 mg Mo/kg DM for 120 d, and liver Cu concentrations in control cattle decreased from 262 mg Cu/kg DM to 11 mg Cu/kg DM. After feeding 6 mg Mo/kg DM for an additional 28 d, the relative bioavailability of CuGly was 150% compared to $CuSO_4$ (Hansen et al., 2008). The difference between bioavailability determined by Hansen et al. (2008) and the present study may be partially due to the greater liver Cu status of steers in the present study. The study conducted by Hansen et al. (2008) utilized steers that had an overall average liver Cu concentration of 256 mg Cu/kg DM, compared to 374 mg Cu/kg DM in the present study. Steers in the present study did not reach deficient status, while those in Hansen et al. (2008) did. Further, Hansen et al. (2008) fed initial treatments for 30 d longer than the present study, providing more time to deplete liver Cu stores to yield increased Cu absorption rates.

When considering the differences in initial Cu status between Hansen et al. (2008) and the present study, the greater bioavailability seen in Hansen et al. (2008) may indicate that organic Cu sources such as CuGly may be more bioavailable and thus better able to replete Cu status in Cu deficient steers, whereas $CuSO_4$ is likely adequate in steers that are not Cu deficient. In practice, however, producers rarely supplement solely organic sources of trace minerals, as 55% of consulting feedlot nutritionists recommend a blend of organic and inorganic trace minerals (Samuelson et al., 2016). Despite this, minimal research has been conducted to determine the optimal combination of organic and inorganic Cu blends to support adequate Cu status.

Further, differences in basal diet Cu concentrations may contribute to variation in Cu bioavailability results. As newer grain hybrids are bred for increased yield, trace mineral concentrations become more dilute. Vyn and Tollenaar (1998) found a significant decrease in Cu concentrations of corn grain in hybrids from the 1960s to hybrids from the 1980s, as breeding for larger grain sizes over time primarily increases carbohydrate content while other nutrients remain constant. This has resulted in dilution of trace minerals

in grain over time. Therefore, even if diet compositions are identical between studies, there may still be variation in the total amount of Cu being fed, especially if the studies occurred at different points in time or geographical locations. Although the rumen solubility of Cu provided by the basal diet was not examined in the present study, it is possible that modern basal diets provide less ruminally available Cu than those from several decades ago. This results in less Cu available for thiomolybdate binding in the rumen, increasing the likelihood of thiomolybdate absorption and systemic Cu antagonism.

In summary, Cu from CuGly was similarly bioavailable relative to $CuSO_4$ when fed in the presence of 2 mg of Mo/kg of DM and 0.3% S. Liver Cu concentration of supplemented treatments decreased between 43% and 65% in the presence of dietary antagonists. Although steers in the present study did not become deficient in liver Cu based on Kincaid (1999), the decrease in liver Cu concentrations across treatments demonstrates the importance of being aware of antagonistic pressure in the diet. Results of the present study, in tandem with the variation in results between other experiments evaluating chelated Cu sources, warrant further research on the impact of initial liver Cu concentrations and the amount of time steers are fed dietary antagonists on the bioavailability of Cu from different sources.

Acknowledgments

This study was funded by Phibro Animal Health Corporation (Teaneck, NJ).

Conflict of interest statement

None declared.

LITERATURE CITED

Engle, T. E., and J. W. Spears. 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J. Anim. Sci. 78:2446–2451. doi:10.2527/2000.7892446x

Gould, L., and N. R. Kendall. 2011. Role of the rumen in copper and thiomolybdate absorption. Nutr. Res. Rev. 24:176–182. doi:10.1017/S0954422411000059

Hansen, S. L., P. Schlegel, L. R. Legleiter, K. E. Lloyd, and J. W. Spears. 2008. Bioavailability of copper from copper glycinate in steers fed high dietary sulfur and molybdenum. J. Anim. Sci. 86:173–179. doi:10.2527/jas.2006-814

Kegley, E. B., and J. W. Spears. 1994. Bioavailability of feed-grade copper sources (oxide, sulfate, or lysine) in growing cattle. J. Anim. Sci. 72:2728–2734. doi:10.2527/1994.72102728x

Kincaid, R. L. 1999. Assessment of trace mineral status of ruminants: a review. Proc. Am. Soc. Anim. Sci. 77:1–10. doi:10.2527/jas2000.77e-suppl1x

Kincaid, R. L., R. M. Blauwiekel, and J. D. Cronrath. 1986. Supplementation of copper as copper sulfate or copper proteinate for growing calves fed forages containing molybdenum. J. Dairy Sci. 69:160–163. doi:10.3168/jds.s0022-0302(86)80381-2

Liu, L., J. Qi, Z. Yang, L. Peng, and C. Li. 2012. Low-affinity copper transporter CTR2 is regulated by copper-sensing transcription factor Mac1p in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 420:600–604. doi:10.1016/j.bbrc.2012.03.040

Messersmith, E. M., E. K. Niedermayer, K. J. Thornton, G. I. Crawford, and S. L. Hansen. 2021. Zinc supplementation strategies in feedlot heifers receiving an extended-release implant or an aggressive re-implant program. J. Anim. Sci. 99:1–8. doi:10.1093/jas/skab212

National Academies of Sciences, Engineering, and Medicine (NASEM). 2016. Nutrient requirements of beef cattle. 8th rev ed. Washington (DC): The National Academies Press. doi:10.17226/19014

Phibro Animal Health Corporation. 2024. GemStone Cu 240 – Copper Amino Acid Complex. [accessed March 18, 2024]. https://www.pahc.com/wp-content/uploads/2022/12/GS031116_Cu_MineralSheet.pdf

Price, J., and J. K. Chesters. 1985. A new bioassay for assessment of copper availability and its application in a study of the effect of molybdenum on the distribution of available Cu in ruminant digesta. Br. J. Nutr. 53:323–336. doi:10.1079/BJN19850040

Rabiansky, P. A., L. R. McDowell, J. Velasquez-Pereira, N. S. Wilkinson, S. S. Percival, F. G. Martin, D. B. Bates, A. B. Johnson, T. R. Batra, and E. Salgado-Madriz. 1999. Evaluating copper lysine and copper sulfate sources for heifers. J. Dairy Sci. 82:2642–2650. doi:10.3168/jds.S0022-0302(99)75521-9

Richter, E. L., M. E. Drewnoski, and S. L. Hansen. 2012. Effects of increased dietary sulfur on beef steer mineral status, performance, and meat fatty acid composition. J. Anim. Sci. 90:3945–3953. doi:10.2527/jas.2011-4512

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Löest. 2016. Nutritional recommendations of feedlot consulting nutritionists: the 2015 New Mexico State and Texas Tech University survey. J. Anim. Sci. 94:2648–2663. doi:10.2527/jas.2016-0282

Suttle, N. 2010. Mineral nutrition of livestock. 4th ed. CABI Publishing, Wallingford, UK.

Suttle, N. F. 1974. Recent studies of the copper-molybdenum antagonism. Proc. Nutr. Soc. 33:299–305. doi:10.1079/pns19740053

Vyn, T. J., and M. Tollenaar. 1998. Changes in chemical and physical quality parameters of maize grain during three decades of yield improvement. Field Crops. Res. 59:135–140. doi:10.1016/S0378-4290(98)00114-2

Ward, J. D., J. W. Spears, and E. B. Kegley. 1993. Effect of copper level and source (copper lysine vs copper sulfate) on copper status, performance, and immune response in growing steers fed diets with or without supplemental molybdenum and sulfur. J. Anim. Sci. 71:2748–2755. doi:10.2527/1993.71102748x

Wittenburg, K. M., R. J. Boila, and M. A. Shariff. 1990. Comparison of copper sulfate and copper proteinate as copper cousrces for copper-depleted steers fed high molybdenum diets. Can. J. Anim. Sci. 70:895–904. doi:10.4141/cjas90-108