Relative bioavailability of organic and hydroxy copper sources in growing steers fed a high antagonist diet¹

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ABSTRACT: To assess relative bioavailability (RBV) of hydroxy and organic Cu sources compared with CuSO₄ in steers fed a high Cu antagonist diet, 84 steers were stratified by BW to pens randomly assigned to dietary treatments for 90 d. Steers received a common corn silage-based diet supplemented with Cu antagonists (diet analyzed 0.25% S; 6.8 mg Mo/kg DM). Supplemental (SUPP) Cu treatments included: control (CON; no SUPP Cu), low or high inorganic (ING5 or ING10; 5 or 10 mg Cu/kg DM from CuSO₄; Old Bridge Chemical Inc., Old Bridge, NJ, USA), low or high organic (ORG5 or ORG10; 5 or 10 mg Cu/kg DM from Cu lysine; CuPlex 100, ZinPro Corp., Eden Prairie, MN), and low or high hydroxy (HYD5 or HYD10; 5 or 10 mg Cu/kg DM; IntelliBond C^{II}, Micronutrients USA LLC, Indianapolis, IN). Body weights were recorded on days -7, -6, 28, 56, 84, and 85, and plasma samples collected on days -7, 28, 56, and 85. Liver samples were collected to start and end the trial. Data were analyzed using the mixed procedure of SAS and the model included treatment with initial liver Cu values used as a covariate in analysis of final liver Cu. Contrast statements were used to separate treatment means: 0 vs. 5 mg SUPP Cu/kg DM, 0 vs. 10 mg SUPP Cu/kg DM, 5 vs. 10 mg

SUPP Cu/kg DM, HYD vs. ORG, HYD vs. ING, and ORG vs. ING. Initial liver Cu concentrations were similar across all treatment comparisons ($P \geq 0.22$). Final liver Cu concentrations were lesser in CON compared with either 5 or 10 mg Cu/kg DM ($P \leq 0.001$). Final liver concentrations were lesser in ORG compared with HYD and ING ($P \leq 0.009$), but HYD was similar to ING ($P = 0.14$). There was a treatment $\times$ time interaction ($P \leq 0.001$) for plasma Cu concentrations where CON exhibited a rapid decline in plasma Cu, steers receiving 5 mg SUPP Cu/kg DM were decreased to a greater extent in ORG, and steers supplemented with 10 mg Cu/kg DM did not differ at the end of the trial. Assessment of RBV was conducted for liver and plasma Cu concentrations using a slope-ratio assay in the GLM procedure. The RBV of Cu tended ($P = 0.07$) to be increased in HYD (112%) compared with ING (100%) for liver Cu values, but RBV was similar for all other source comparisons based on liver and plasma Cu values ($P \geq 0.22$). These data suggest in steers fed high antagonist diets hydroxy Cu may be more available. Based on plasma and liver Cu concentrations, supplementation of 10 mg Cu/kg DM is needed to maintain Cu status in cattle fed diets high in S and Mo.

Key words: bioavailability, cattle, copper, molybdenum, sulfur

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INTRODUCTION

Certain trace minerals (TM) including Cu are essential in livestock diets, and deficiencies result in decreased animal health and performance (Hostetler et al., 2003). Copper is critical

in growth processes such as collagen and elastin formation (Mills et al., 1976), and as a catalytic component of enzymes including Cu–Zn superoxide dismutase (Prohaska, 1991). Increased inclusion of ethanol coproducts in livestock diets has raised concern about greater dietary S concentrations resulting in decreased TM absorption due to ruminal antagonisms (Drewnoski et al., 2014). Sulfide in the rumen may bind Cu, and increasing dietary Mo supports formation of thiomolybdates creating insoluble complexes with Cu, keeping Cu bound even under acidic conditions (Spears, 2003). Providing TM in a form that is less susceptible to dietary antagonisms may increase TM absorption. Hydroxy TM have low solubility in water, whereas inorganic CuSO_4 is highly soluble in water (Cromwell et al., 1998; Cao et al., 2000), suggesting CuSO_4 may be more susceptible to ruminal antagonisms. Hydroxy TM are soluble in slightly acidic conditions (Cao et al., 2000), whereas organic Cu lysine is soluble in water and acidic conditions (Guo et al., 2001). Spears et al. (2004) determined relative bioavailability (RBV) of tribasic Cu chloride (hydroxy source) to be 196% compared with CuSO_4 based on liver Cu concentrations in growing steers fed a high antagonist diet. However, little work has been performed to assess the RBV of Cu from hydroxy sources compared with organic and inorganic sources in ruminants; thus, the objective of this study was to determine the RBV of Cu from basic Cu chloride or Cu lysine compared with CuSO_4 based on liver and plasma Cu concentrations of steers. It was hypothesized that Cu from basic Cu chloride and Cu lysine would have increased RBV compared with Cu from CuSO_4 based on plasma and liver Cu concentrations in growing steers fed a high antagonist diet.

MATERIALS AND METHODS

All experimental procedures were approved by the Iowa State University institutional animal care and use committee (log number 12-16-8407-B).

Experimental Design and Sample Collection

Eighty-four Angus crossbred steers (initial BW: 296 ± 22.7 kg) were housed at the Iowa State University Beef Nutrition Research Center (Ames, IA). Steers were housed in pens equipped with GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) to allow for measurement of individual feed intake ($n = 6$ steers per pen; 1 bunk per pen). Pens were equipped with

automatic waterers, a water analysis was collected and analyzed by Midwest Laboratories (Omaha, NE), and the Cu concentration was found to be 0.02 mg/L, and sulfate was 112 mg/L. On day –7, steers were weighed prior to feeding, dewormed with Ivomec Eprinex Pour-On (Merial Animal Health, Duluth, GA), vaccinated with Bovi-shield GOLD 5 (Zoetis Inc., New York, NY), implanted with Component E-S implant (Elanco Animal Health, Greenfield, IN), and received unique visual and electronic identification tags. On day –6, steers were again weighed prior to feeding, and the average of the days –7 and –6 weights were used to stratify steers to 1 of 7 treatments ($n = 12$ per treatment).

All steers were fed a common corn silage-based total mixed ration (TMR; Table 1) throughout the 90-d trial. The TMR included a TM premix that was formulated to provide supplemental Co, Mn, Se, Zn, and I from inorganic sources at requirement (NASEM, 2016), supplemental S (as CaSO_4) at 0.16% diet DM (to achieve ~0.3% total dietary S), and supplemental Mo as (Na_2MoO_4) at 5 mg/kg DM. Nutrient composition of the diet is shown in Table 1, the TMR analyzed to contain 0.25% S and 6.8 mg Mo/kg DM in total.

Table 1. Common TMR formulation

Item	DM, %
Corn silage	84.15
Dried distillers grains	5.85
Supplemental Cu premix ¹	5
Basal ²	5
Analyzed composition ³	
DM ⁴	41.19, 31.80
OM	92.55
NDF	36.31
CP	11.99
Ether extract	4.01
S	0.25
Cu mg/kg diet DM	4.5
Mo mg/kg diet DM	6.8

¹Supplemental Cu premix provided 5% diet DM as distillers grains and contributed 0, 5, or 10 mg supplemental Cu/kg of complete diet from either CuSO_4 , Cu lysine, or basic Cu chloride.

²Basal provided per kilogram of diet DM: 0.15 mg Co (cobalt carbonate), 20 mg Mn (manganese sulfate), 0.1 mg Se (sodium selenite), 30 mg Zn (zinc sulfate), 5 mg Mo (sodium molybdate), 0.5 mg I (calcium iodate). Remaining contributed (as % of total diet DM): dried distillers grains 2.85%, limestone 0.5%, vitamin A and E premix 0.11%, calcium sulfate 0.9%, salt 0.31%, urea 0.3%, Rumensin 90 0.008%.

³DM and Cu were determined by the Hansen Laboratory, and all other values were determined by Dairyland Laboratories.

⁴DM values are reported as the average of weekly DM samples with silage source 1 and after the change to silage source 2 that occurred the week of 30 May 2017.

Dietary Cu treatments included 1) control (CON), common TMR with no supplemental Cu, 2) low inorganic (ING5), common TMR plus 5 mg Cu/kg DM supplemental Cu from CuSO_4 , 3) high inorganic (ING10), common TMR plus 10 mg Cu/kg DM supplemental Cu from CuSO_4 , 4) low organic (ORG5), common TMR plus 5 mg Cu/kg DM supplemental Cu from Cu lysine, 5) high organic (ORG10), common TMR plus 10 mg Cu/kg DM supplemental Cu from Cu lysine, 6) low hydroxy (HYD5), common TMR plus 5 mg Cu/kg DM supplemental Cu from basic Cu chloride, and 7) high hydroxy (HYD10), common TMR plus 10 mg Cu/kg DM supplemental Cu from basic Cu chloride. Treatments were assigned randomly to pens and steers were stratified to pens and treatments by initial BW. Three steers, one each from HYD10, ORG10, and ING10, were removed from the trial due to issues unrelated to treatment (final $n = 11$ for these 3 treatments). Inorganic Cu was provided as CuSO_4 pentahydrate (Old Bridge Chemical Inc., Old Bridge, NJ), organic Cu as Cu lysine (CuPlex 100, ZinPro Corp., Eden Prairie, MN), and hydroxy Cu as basic Cu chloride (IntelliBond C^{II}, Micronutrients USA LLC, Indianapolis, IN). Supplemental Cu was provided in a Cu premix at 5% of diet DM. Copper analysis of TMR samples was conducted for 4 time periods across the trial, representing months or major ingredient changes. Across the trial average Cu concentrations (mg/kg DM) were as follows: CON (4.5), ING5 (12.0), ORG5 (9.9), HYD5 (10.8), ING10 (18.5), ORG10 (13.6), and HYD10 (16.2).

Steers were weighed prior to feeding on 2 consecutive days near the start (days -7, -6) and end of the trial (days 84, 85) and were weighed every 28 d throughout the trial and a 4% shrink was applied to all BW data. Blood was collected prior to feeding via jugular venipuncture into 7-mL trace element K_2EDTA tubes (Becton Dickinson and Company, Franklin Lakes, NJ) on days -7 (initial), 28, 56, and 85 (final) for the determination of plasma Cu concentrations. Blood was stored on ice until arrival at the laboratory, centrifuged at $1,200 \times g$ for 10 mins at 4 °C, and plasma was stored at -20 °C until further analysis. Liver biopsies were collected from all steers approximately 2 h post-feeding, and because of the large number of animals to sample, biopsies were conducted over a 3-d period (days -3, -2, -1 for initial and days 88, 89, 90 for final), with unequal numbers of treatments biopsied each day. On day -3, one pen of ING10, HYD10, ORG5, and

HYD5 were biopsied, on day -2, 2 pens of ING 5 were biopsied, along with one pen each of ORG10 and CON. One pen each of CON, HYD5, HYD 10, ORG5, ORG10, and ING10 were biopsied on day -1. Pens were biopsied in the same order on days 88, 89, and 90, so that the time between biopsies would be the same for all steers. Biopsies were conducted using the methods of [Engle and Spears \(2000\)](#), and samples were placed on ice for transport to the laboratory.

Tissue and Feed Analysis

Total mixed ration samples were collected on a weekly basis and were dried in a forced-air oven at 70 °C for 48 h for DM determination. Dried TMR samples were ground to fit through a 2-mm screen in a Retsch ZM 100 grinding mill (Retsch GmbH, Haan, Germany). Dried and ground samples were composited into monthly composite samples by treatment. Dry matter intake was calculated using as-fed intake data from the GrowSafe system and corrected for DM content based on weekly TMR sample analysis. Dried weekly TMR were ground and composited by treatment into monthly composite samples for TM analysis. Average daily gain was determined for each monthly period (days 0 to 28, days 29 to 55, and days 56 to 84) and the overall feeding period (days 0 to 84).

On arrival at the laboratory, fresh liver samples were dried in a forced-air oven at 70 °C for approximately 1 wk, until all samples were completely dry. Dried liver and TMR composite samples were acid digested (CEMS MARSXpress, Matthews, NC) with TM grade nitric acid and diluted to 20% nitric acid with deionized water. Plasma samples were prepared for TM analysis by the methods of [Pogge and Hansen \(2013\)](#).

Total mixed ration, plasma, and liver samples were analyzed for Cu content via inductively coupled plasma atomic emissions spectrometry (Optima 7000 DV, PerkinElmer, Waltham, MA) as described by [Pogge and Hansen \(2013\)](#). Each run included a liver (National Institute of Standards and Technology, Gaithersburg, MD) or serum (UTAK Laboratories Inc., Valencia, CA) to verify instrument accuracy. The interassay CV for serum standards was 7.6%, and the intraassay was $\leq 1.67\%$. The interassay CV for liver standards was 4.7%, and the intra assay CV was $\leq 1.72\%$. Actual Cu intake of each steer was determined by multiplying DMI by analyzed Cu content of the respective dietary TMR for the appropriate period.

Statistical Analysis

Liver and plasma Cu concentrations and performance data were analyzed using the mixed procedure of SAS (SAS version 9.4, SAS Inst. Inc., Cary, NC). Steer was utilized as the experimental unit ($n = 12$ per treatment; with the exception of ING10, ORG10, and HYD10 where $n = 11$). The model included the fixed effect of treatment. Initial liver Cu concentrations were analyzed as a covariate for final liver Cu, and initial and final liver Cu concentrations were log transformed to account for homogeneity of variances. Six single df contrasts were constructed: 1) 0 mg supplemental Cu/kg of DM vs. 5 mg supplemental Cu/kg of DM, 2) 0 mg supplemental Cu/kg of DM vs. 10 mg supplemental Cu/kg DM, 3) 5 mg supplemental Cu/kg DM vs. 10 mg supplemental Cu/kg DM, 4) HYD vs. ORG, 5) HYD vs. ING, and 6) ORG vs. ING.

Plasma Cu data were analyzed using repeated measures with steer as the experimental unit, the model included the fixed effects of treatment, time of sampling, and the interaction. Based on the lowest Akaike information corrected criterion, unstructured was selected as the covariance structure. Initial plasma Cu values were used as a covariate for analysis of subsequent sampling days. All plasma data were square root transformed to account for homogeneity of variances. Back transformed means and SEM for liver and plasma Cu data are reported.

Plasma and liver Cu concentrations were used to determine RBV of basic Cu chloride and Cu lysine relative to CuSO_4 using the methods described by Hansen et al. (2008). Briefly, final liver Cu concentrations and days 85 plasma Cu concentrations were regressed against daily Cu intake determined by TMR analysis during the 90-d trial period. Initial plasma and liver Cu concentrations were used as covariates in the final models as appropriate. Assumptions for the slope-ratio assay were checked for validity as described by Littell et al. (1997). The final models for the estimation of RBV from both plasma and liver included initial tissue Cu concentrations as a covariate, total analyzed Cu intake nested within source, and an XO indicator variable to meet the requirements for equality of intercepts for each Cu source. All data were examined for outliers using Cook's D statistics. One steer each from ING5, ORG5, and CON was removed from plasma analysis due to plasma Cu values that were higher than physiologically expected. Removal of these steers was needed to improve

fit of the model and meet assumptions of normality. Significance was declared at $P \leq 0.05$, and tendencies were declared at $0.06 \leq P \leq 0.10$.

RESULTS

Performance Measures and DMI

Data regarding steer BW, ADG, and DMI are presented in Table 2. Steer BW did not differ at any point in the trial ($P \geq 0.28$), nor did ADG differ between treatments for the periods of days 0 to 28 or 56 to 84 ($P \geq 0.13$). However, ADG of steers receiving 10 mg of supplemental Cu/kg of DM from days 29 to 55 was greater ($P = 0.03$) than CON steers and tended ($P = 0.07$) to be greater than steers receiving 5 mg of supplemental Cu/kg of DM. When evaluating ADG across the entire trial period steers consuming 10 mg of supplemental Cu/kg of DM showed a tendency ($P = 0.08$) for improved ADG compared with steers consuming 5 mg of supplemental Cu/kg of DM. Steers fed ORG Cu had a tendency ($P = 0.09$) for decreased overall ADG compared with steers consuming ING Cu. This decrease in performance was probably driven by lesser DMI by ORG compared with ING ($P = 0.04$). There was a tendency for CON to have lesser DMI than steers consuming 10 mg of supplemental Cu/kg DM ($P = 0.10$).

Liver and Plasma Cu Concentrations and Cu Intake

Initial and final liver Cu concentrations and Cu intake data are reported in Table 3. Steers in the present study arrived with moderate Cu status (average = 55.6 mg/kg DM), and initial liver Cu concentrations were not different among treatments ($P \geq 0.22$). Final liver Cu concentrations were affected by dietary Cu treatment. Control steers had lesser liver Cu concentrations than steers consuming either 5 or 10 mg supplemental Cu/kg DM ($P < 0.001$). Steers consuming 5 mg Cu/kg DM had lesser final liver concentrations compared with those receiving 10 mg Cu/kg DM ($P < 0.001$). Final liver Cu concentrations did not differ between ING and HYD steers ($P = 0.14$), and both ING and HYD steers had greater liver Cu than ORG steers ($P \leq 0.009$).

By design Cu intake was lesser in CON compared with steers receiving 5 or 10 mg of supplemental Cu/kg of DM ($P \leq 0.001$), and steers receiving 5 mg supplemental Cu/kg of DM had lesser Cu intake compared with those receiving 10 mg supplemental Cu/kg of DM ($P \leq 0.001$).

Table 2. Influence of supplemental Cu concentration and source on steer growth performance

Table 1. Effect of treatment on BW and ADG of growing steers														
Treatment ¹			Contrast ²						Contrast ³					
Item	CON	ING5	ORG5	HYD5	ING10	ORG10	HYD10	SEM	0 vs. 5	0 vs. 10	5 vs. 10	HYD vs. ORG	HYD vs. ING	ORG vs. ING
BW, kg														
Initial ^{3,4}	284	285	282	283	281	283	283	6.76	0.96	0.99	0.97	0.84	0.99	0.85
Day 28 ⁴	348	342	342	344	353	343	346	8.30	0.55	0.92	0.72	0.71	0.80	0.53
Day 55 ⁴	393	394	390	392	404	395	401	9.30	0.90	0.52	0.36	0.62	0.79	0.45
Final ^{4,5}	433	436	428	430	446	437	442	9.80	0.91	0.42	0.28	0.70	0.58	0.35
ADG, kg/d														
Initial to 28 d ⁶	1.90	1.69	1.76	1.81	2.10	1.65	1.87	0.123	0.28	0.85	0.44	0.29	0.63	0.13
29 to 55 d	1.66	1.94	1.78	1.75	1.89	1.91	2.05	0.112	0.22	0.03	0.07	0.62	0.89	0.53
56 to 84 d	1.37	1.44	1.32	1.34	1.47	1.46	1.38	0.087	0.98	0.46	0.44	0.76	0.26	0.41
Overall	1.64	1.66	1.61	1.62	1.81	1.65	1.75	0.067	0.90	0.20	0.08	0.39	0.38	0.09
DMI, kg/d	8.80	9.84	8.85	9.39	9.84	9.25	9.44	0.348	0.17	0.10	0.63	0.35	0.23	0.04

¹Treatment: CON = common TMR with no supplemental Cu, ING5 = common TMR plus 5 mg Cu/kg DM from CuSO₄, ORG5 = common TMR plus 5 mg Cu/kg DM from Cu lysine, HYD5 = common TMR plus 5 mg Cu/kg DM from basic Cu chloride, ING10 = common TMR plus 5 mg Cu/kg DM from CuSO₄, ORG10 = common TMR plus 10 mg Cu/kg DM from Cu lysine, HYD10 = common TMR plus 10 mg Cu/kg DM from basic Cu chloride.

²Contrast: Control vs. 5 mg Cu/kg DM treatments, Control vs. 10 mg Cu/kg DM treatments, 5 mg Cu/kg DM treatments vs. 10 mg Cu/kg DM treatments, HYD supplemented vs. ORG supplemented, HYD supplemented vs. ING supplemented, ING supplemented vs. ORG supplemented.

³Initial BW reported as the average of BW recorded on days -6 and -7.

⁴All BW data have had a 4% shrink applied.

⁵Final BW reported as the average of BW recorded on days 84 and 85.

⁶Calculated as day 28 BW – initial BW divided by 34 d.

Table 3. Influence of supplemental Cu concentration and source on liver Cu concentrations of steers

Table 1. Effect of dietary organic acids on the growth performance, carcass characteristics, and organ weights of broilers.														
Treatment ¹			Contrast ²											
Item	CON	ING5	ORG5	HYD5	ING10	ORG10	HYD10	SEM	0 vs. 5	0 vs. 10	5 vs. 10	HYD vs. ORG	HYD vs. ING	ORG vs. ING
Liver Cu ³ , mg/kg DM														
Initial	56.2	50.6	42.6	39.7	53.1	44.2	54.1	4.47	0.22	0.57	0.80	0.71	0.54	0.32
Final ⁴	6.13	18.5	11.6	14.4	83.4	41.9	70.0	5.42	0.001	0.001	0.001	0.009	0.14	0.001
Cu intake ⁵ , g	3.35	10.6	7.87	8.68	15.6	11.9	13.5	0.376	0.001	0.001	0.001	0.002	0.001	0.001

¹Treatment: CON = common TMR with no supplemental Cu, ING5 = common TMR plus 5 mg Cu/kg DM from CuSO₄, ORG5 = common TMR plus 5 mg Cu/kg DM from Cu lysine, HYD5 = common TMR plus 5 mg Cu/kg DM from basic Cu chloride, ING10 = common TMR plus 5 mg Cu/kg DM from CuSO₄, ORG10 = common TMR plus 10 mg Cu/kg DM from Cu lysine, HYD10 = common TMR plus 10 mg Cu/kg DM from basic Cu chloride.

²Contrast: Control vs. 5 mg Cu/kg DM treatments, Control vs. 10 mg Cu/kg DM treatments, 5 mg Cu/kg DM treatments vs. 10 mg Cu/kg DM treatments, HYD supplemented vs. ORG supplemented, HYD supplemented vs. ING supplemented, ING supplemented vs. ORG supplemented.

³Liver Cu concentrations were log transformed, and means and SEM shown here have been back calculated from log-transformed values.

⁴Final liver Cu analysis included initial liver Cu concentration as a covariate.

⁵Total Cu intake per steer over the entire trial.

Steers consuming hydroxy Cu had greater Cu intake compared with ORG ($P \leq 0.002$), whereas ING had greater Cu intake compared with ORG and HYD ($P \leq 0.001$).

Plasma Cu concentrations across the trial are presented in Fig. 1. There was a treatment $\times$ time effect ($P < 0.001$) where though steers started with similar day 0 plasma Cu concentrations (average = 0.82 mg/L; $P = 0.20$), by day 28 CON steers had lesser plasma Cu concentrations compared

with all other treatments ($P \leq 0.001$), and CON plasma Cu concentrations progressively decreased with each subsequent sampling date ($P \leq 0.001$). In general, steers receiving supplemental Cu maintained plasma Cu concentrations well above CON throughout the trial. On day 85, steers receiving 5 mg of supplemental Cu/kg of DM from either ORG or HYD had decreased plasma Cu compared with day 28 ($P \leq 0.007$). Steers receiving 5 mg of supplemental Cu/kg of DM from the ING source,

or those receiving 10 mg of supplemental Cu/kg of DM from ING or ORG, had similar plasma Cu concentrations throughout the trial ($P \geq 0.23$).

Liver and Plasma Bioavailability

Slopes from the multiple linear regression analysis as well as RBV calculations can be found in Table 4. Using final liver Cu concentrations, the RBV of HYD tended to be greater than ING ($P = 0.07$; HYD RBV = 112%). The RBV of ORG was similar to ING ($P = 0.65$) based on liver Cu concentrations. Slopes for liver Cu were not different between HYD and ORG ($P = 0.22$). There was no difference between the slopes of the different Cu sources produced from the linear regression of day 85 plasma concentration on daily Cu intake ($P \geq 0.91$).

Figure 1. Plasma Cu concentrations of steers supplemented with 0, 5, or 10 mg Cu/kg DM from CuSO_4 (ING), Cu lysine (ORG), basic Cu chloride (HYD) fed a corn silage-based diet containing supplemental S and Mo. Plasma Cu concentrations analyzed as a repeated measure with initial plasma Cu concentration used as a covariate in the analysis. Data shown have been square root transformed for analysis, and back transformed means and SEM are presented. Plasma Cu concentrations exhibited a treatment $\times$ time interaction ($P \leq 0.001$).

DISCUSSION

The objective of this study was to determine whether Cu from basic Cu chloride (IntelliBond C^{II}; HYD) would be more effective in maintaining or preventing a decline in Cu status than Cu lysine (CuPlex 100; ORG) and CuSO_4 (ING) in steers consuming a diet containing the Cu antagonists S and Mo. Increased S inclusion from ethanol coproducts in feedlot diets can lead to decreased absorption of TM including Cu (Drewnoski et al., 2014). Additional sources of S include molasses, and sulfates found in drinking water. Forages may contain a significant amount of Mo; however, corn silage typically contains low concentrations of Mo (Suttle, 2010). The present study used a corn silage-based diet, without high inclusions of ethanol coproducts; therefore, S was supplemented to provide industry relevant concentrations in the diet (Drewnoski et al., 2014) and Mo was supplemented as a further Cu antagonist (Spears et al., 2004). Because the S and Mo antagonism of Cu occurs initially in the rumen, it is of particular interest to provide supplemental Cu in a form that can overcome these antagonisms and is available for use by the animal.

In the present study, initial liver and plasma Cu concentrations were similar and adequate across all treatments (Underwood and Suttle, 1999). Steers in the present study consuming 0 mg supplemental Cu/kg DM were Cu deficient, whereas steers receiving 5 mg supplemental Cu/kg DM were marginally Cu deficient by the end of the study; however, steers consuming 10 mg supplemental Cu/kg DM were adequate (Underwood and Suttle, 1999). This suggests that regardless of source, inclusions below NASEM (2016) recommendations are inadequate to maintain liver Cu status in cattle consuming diets high in Cu antagonists. Hansen et al.

Table 4. Estimated relative bioavailability of Cu sources in steers fed high antagonist diets, based on multiple linear regression of Cu indices on total Cu intake (g)

Contrast ¹						
Cu indices	Source ²	Slope $\pm$ SE	Relative bioavailability, %	HYD vs. ORG	HYD vs. ING	ING vs. ORG
Liver ³	ING	8.586 $\pm$ 0.9386	100	0.22	0.07	0.65
	ORG	8.879 $\pm$ 1.2150	103			
	HYD	9.646 $\pm$ 1.0811	112			
Plasma ⁴	ING	0.0181 $\pm$ 0.00614	100	0.97	0.91	0.96
	ORG	0.0183 $\pm$ 0.00792	101			
	HYD	0.0185 $\pm$ 0.00713	102			

¹Contrasts: HYD supplemented vs. ORG supplemented, HYD supplemented vs. ING supplemented, ING supplemented vs. ORG supplemented.

²Sources include ING = Cu from CuSO_4 , ORG = Cu from Cu lysine, and HYD = Cu from basic Cu chloride.

³Liver data utilized from liver biopsies taken at the end of the trial, and initial liver Cu concentrations were used as a covariate.

⁴Plasma data utilized were collected on day 85, and initial plasma Cu concentrations were used as a covariate.

(2008) similarly found that 10 mg supplemental Cu/kg DM was necessary for steers fed diets of 0.24% S and 2 mg supplemental Mo/kg DM to maintain adequate Cu status. These findings reiterate that dietary antagonists should be assessed when developing Cu supplementation strategies for cattle.

As foreshadowed by changes in liver Cu concentrations, plasma Cu concentrations fell rapidly in steers receiving no supplemental Cu suggesting that homeostatic mechanisms that control plasma Cu concentrations were overwhelmed when dietary antagonists were included in the diet, resulting in lesser available Cu in circulation. Liver Cu stores can be released to maintain plasma Cu status until liver Cu concentrations fall below 30 mg Cu/kg DM (Mills, 1987), and in the present study, final liver Cu concentrations for steers receiving 0 or 5 mg supplemental Cu/kg DM were below 30 mg Cu/kg DM. Although animals consuming 5 mg supplemental Cu/kg DM were able to maintain plasma Cu status within the acceptable range defined by Underwood and Suttle (1999), it is likely that the continued feeding of a high antagonist diet would have depleted plasma Cu stores in those animals. However, animals consuming 10 mg supplemental Cu/kg DM were able to maintain liver Cu concentrations above 30 mg Cu/kg DM and thus plasma Cu concentrations within the acceptable range defined by Underwood and Suttle (1999).

In this study, a slope-ratio assay was performed to determine the RBV of Cu from basic Cu chloride or Cu lysine compared with CuSO_4 based on either liver or plasma Cu concentrations regressed against Cu intake. The tendency for an increase in RBV of HYD Cu (112%) compared with ING (set at 100%) based on liver Cu concentrations from the end of the feeding period suggests basic Cu chloride may be more efficiently absorbed and incorporated into liver Cu stores. Spears et al. (2004) observed greater RBV of Cu in steers supplemented with tribasic Cu chloride compared with CuSO_4 , where tribasic Cu chloride supplementation had 196% RBV compared with CuSO_4 . It is unclear why the RBV of tribasic Cu chloride in Spears et al. (2004) was so much greater than that determined in the present study as the diet and length of study were quite similar between the 2 studies. Steers utilized by Spears et al. (2004) in their experiment one had greater initial liver Cu status compared with the initial liver Cu concentrations of steers used in the present study. The effect of initial Cu status on determination of RBV of Cu sources is not well studied; however, when Cu-depleted steers were utilized in a 21-d experiment in the absence of dietary antagonists,

there was no difference in RBV of tribasic Cu chloride and CuSO_4 (Spears et al., 2004). This suggests that RBV between Cu sources can differ when dietary antagonists are included in the diet. In the present study, total Cu intake was lesser in CON compared with Cu supplemented treatments, while being greater in HYD and ING supplemented animals compared with ORG. It is unlikely that differences in Cu intake affected the RBV calculations as individual animal Cu intake was accounted for in this calculation; however, further research examining the effects of Cu intake, initial Cu status, and antagonist supplementation strategy on RBV of Cu sources would be valuable. Diet type may also affect availability of Cu sources. In a study with ~20% distillers grains, steers consuming high S diets supplemented with hydroxy TM (Cu, Mn, Zn) exhibited lesser liver Cu values than those supplemented with sulfates, and it was suggested that ruminally available Cu may be beneficial in preventing absorption of thiomolybdates (Hartman et al., 2017). This suggests that feeding a blend of Cu sources may be more beneficial at maintaining Cu status in the presence of high antagonist diets. The steers used by Hartman et al. (2017) were fed a high concentrate diet, which probably resulted in a lesser ruminal pH compared with steers consuming the corn silage-based diet in the present trial. There is currently a limited understanding of how ruminal pH may influence ruminal metabolism of Cu sources. The corn silage-based diet utilized in the present study was supplemented with S and Mo, and further work should be conducted to determine RBV of Cu sources in steers fed coproduct-based diets, while assessing the impacts of initial liver Cu status, length of feeding period, and ruminal pH on RBV of Cu sources.

Relative bioavailability of Cu sources based on plasma Cu concentrations has yielded variable results (Ward et al., 1993; Spears et al., 2004; Hansen et al., 2008). In this study, slope estimates were not different due to source of Cu based on plasma Cu concentrations. Previous studies (Ward et al., 1993; Rabiansky et al., 1999) have shown CuSO_4 and Cu lysine to have similar availabilities when fed with dietary antagonists to both steers (5 mg Mo/kg DM, 0.2% S) and heifers (1,000 mg Fe/kg DM, 0.5% S, 5 mg Mo/kg DM). The results of the present study agree with this previous work, where ORG was found to have similar RBV to ING. The strict homeostatic mechanisms by which plasma Cu concentrations are controlled make assessing differences in RBV of Cu sources from plasma Cu concentrations difficult, as these homeostatic

mechanisms must be overwhelmed before differences in RBV can be adequately assessed. In the present study, CON steers receiving no supplemental Cu displayed a dramatic decrease in plasma Cu status, whereas other treatments were able to better maintain plasma Cu status. This suggests that the homeostatic mechanisms were overwhelmed only in the CON steers in the present study.

While not evaluated in the present study, when compared with CuSO_4 , tribasic copper chloride is less soluble in water (Miles et al., 1998; Spears et al., 2004). Following in vitro incubation in rumen fluid Cu lysine exhibited increased solubility compared with CuSO_4 , but rumen fluid samples collected via a stomach tube from steers and heifers fed CuSO_4 or Cu lysine exhibited no difference in ruminally soluble Cu (Kegley and Spears, 1994). Differences in ruminal solubility across TM sources may help explain the tendency for increased RBV of HYD compared with ING in the present study, whereas ORG had a similar RBV compared with ING. In assessing the effects of supplemental Cu concentration and source on steer performance in this study, steers receiving no supplemental Cu tended to have lesser DMI compared with steers receiving 10 mg supplemental Cu/kg of DM. Previous studies have shown variable effects of Cu supplementation on DMI (Spears et al., 2004; Hansen et al., 2008). However, impacts on DMI in steers receiving no supplemental Cu may be dependent on Cu concentration of the unsupplemented diet and the presence of Cu antagonists in the diet. In the present study, DMI was improved in steers receiving ING supplemental Cu compared with those receiving ORG, which may explain the tendency for improved overall ADG in ING vs. ORG steers. Interestingly, although 10 mg supplemental Cu/kg DM was required to maintain adequate liver Cu concentrations in the face of dietary Cu antagonists, minimal effects of dietary Cu concentration on steer ADG were noted outside the tendency for 10 mg supplemental Cu/kg DM to gain better than those receiving 5 mg supplemental Cu/kg DM. Collectively, these data suggest steers were able to maintain adequate growth rates while experiencing moderate Cu deficiency based on liver Cu concentrations, suggesting there is opportunity to refine the Cu requirements of feedlot cattle.

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