Relative Bioavailability of Supplemental Cu Sources as Determined in Growing Steers Fed a High Antagonist Diet

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Summary and Implications

The objective of this study was to evaluate the relative bioavailability of an organic Cu source, Cu lysine and a hydroxy Cu source, basic Cu chloride, relative to an inorganic Cu source, $CuSO_4$. Although initial liver Cu concentrations were similar across all treatments, final liver Cu concentrations were lesser in those cattle receiving 0 ppm vs. 5 or 10 ppm of supplemental Cu. This is to be expected when feeding a diet supplemented with the Cu antagonists S and Mo. However, steers receiving 0 or 5 ppm supplemental Cu had liver Cu concentrations that were either deficient or marginally deficient, regardless of source. This suggests that in cattle consuming high antagonist diets, 10 ppm of supplemental Cu is necessary to maintain liver Cu status. The relative bioavailability of basic Cu chloride tended to be greater (112%) compared to $CuSO_4$, while the relative bioavailability of Cu lysine was similar to that of $CuSO_4$ as determined by liver Cu concentrations. Ultimately, when fed in high antagonist diets basic Cu chloride may be more bioavailable to the ruminant animal then $CuSO_4$.

Introduction

Copper is an essential trace mineral, and is critical for growth processes including collagen and elastin formation, which provide support to growing bones and muscles. Copper is also a component of metalloenzymes, making Cu critical for proper enzyme function. Due to increased inclusion of ethanol co-products in livestock diets, increasing dietary S concentrations have become a concern, due to their ability to limit trace mineral absorption. Sulfide in the rumen can bind Cu, and when dietary Mo is also increased, thiomolybdates form insoluble complexes with Cu.

Providing supplemental Cu in a form that can overcome dietary antagonists is critical for maintaining Cu absorption in animals consuming diets high in Cu antagonists. While inorganic $CuSO_4$ and organic Cu lysine are both soluble in water, basic Cu chloride exhibits low solubility in water. This may suggest that hydroxy trace mineral sources are less susceptible to ruminal antagonisms than inorganic and organic sources. Thus, the objective of this study was to evaluate the relative bioavailability of Cu lysine and basic Cu chloride compared to $CuSO_4$ in growing steers fed a diet high in Cu antagonists.

Materials and Methods

Eighty-four Angus crossbred steers (initial BW: 653 ± 55 lbs) were housed in pens equipped with GrowSafe bunks to allow for determination of individual animal feed intake (n = 6 steers/pen). On d -7 and -6 BW was recorded and the average of the consecutive day weights were used to stratify steers to 1 of 7 treatments (n = 12/treatment). On d -7 steers were dewormed with Ivomex Eprinex Pour-On (Merial Animal Health, Duluth, GA), vaccinated with Bovi-shield GOLD 5 (Zoetic Inc., New York, NY), and implanted with a Component E-S implant (Elanco Animal Health, Greenfield, IN).

All steers were fed a common diet (**Table 1**) throughout the 90 d trial, that was formulated to provide supplemental Co, Mn, Se, Zn, and I from inorganic sources at NASEM recommended requirements, and supplemental S (as $CaSO_4$) at 0.16% of diet DM (to achieve ~0.3% total dietary S), and 5 ppm supplemental Mo (as Na_2MoO_4). The diet analyzed at 0.25% S and 6.8 ppm Mo in total. Dietary Cu treatments included 1) control (**CON**), common diet with no supplemental Cu, 2) low inorganic (**ING5**), common diet plus 5 ppm Cu from $CuSO_4$, 3) high inorganic (**ING10**), common diet plus 10 ppm Cu from $CuSO_4$, 4) low organic (**ORG5**), common diet plus 5 ppm Cu from Cu lysine, 5) high organic (**ORG10**), common diet plus 10 ppm Cu from Cu lysine, 6) low hydroxy (**HYD5**) common diet plus 5 ppm from basic Cu chloride, 7) high hydroxy (**HYD10**) common diet plus 10 ppm from basic Cu chloride.

Blood samples were collected for determination of plasma Cu concentrations on d -7 (initial), 28, 56, and 85 (final). Liver biopsy samples for determination of Cu concentrations, were collected over 3 d to start (d -3, -2, -1) and end (d 88, 89, 90) the trial. Plasma and liver Cu concentrations were determined via inductively coupled plasma atomic emission spectrometry.

Liver and plasma Cu concentrations were analyzed using the Mixed procedure of SAS (SAS version 9.4, SAS Inst. Inc., Cary, NC). Steer was the experimental unit, and the model included the fixed effect of treatment. Initial liver Cu concentrations were used as a covariate in the analysis of final liver Cu concentrations, and liver Cu concentrations were log transformed to account for homogeneity of variances. Contrast statements were developed for comparison of treatment means: 1) 0 ppm supplemental Cu vs. 5 ppm supplemental Cu, 2) 0 ppm supplemental Cu vs. 10 ppm supplemental Cu, 3) 5 ppm

supplemental Cu vs. 10 ppm supplemental Cu, 4) HYD vs. ORG, 5) HYD vs. ING, 6) ORG vs. ING.

Plasma Cu concentrations were analyzed using repeated measures with steer as the experimental unit, and the model included the fixed effects of treatment, time of sampling, and the interaction. Unstructured was used as the covariance structure, and initial plasma Cu concentrations were used as a covariate in the analysis of subsequent sampling dates. All plasma data were square root transformed to account for homogeneity of variances, and back transformed means and SEM are reported.

Plasma and liver Cu concentrations were used to determine RBV of Cu lysine and basic Cu chloride relative to $CuSO_4$. Final liver Cu concentrations and d 85 plasma Cu concentrations were regressed against daily Cu intake determined by diet 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, and the final models 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.

Results and Discussion

Initial liver Cu concentrations were not different among treatments ($P \geq 0.22$); however, steers arrived with moderate Cu status (average = 56 ppm). Final liver Cu concentrations were affected by dietary treatment, with CON having lesser liver Cu concentrations compared to steers consuming either 5 or 10 ppm of supplemental Cu ($P < 0.001$). Steers receiving no supplemental Cu were considered to be Cu deficient, whereas those receiving 5 ppm of supplemental Cu were marginally Cu deficient, and those receiving 10 ppm supplemental Cu were adequate at the completion of the trial. Final liver Cu concentrations did not differ between ING and HYD ($P = 0.14$), but both ING and HYD steers had greater liver Cu than ORG ($P \leq 0.009$). However, these data suggest that regardless of supplemental Cu source, supplementation of Cu below 10 ppm is inadequate for maintaining liver Cu status in cattle consuming high antagonist diets.

Copper intake was lesser in CON vs. steers receiving 5 or 10 ppm supplemental Cu, and was lesser in steers receiving 5 ppm vs. 10 ppm supplemental Cu ($P \leq 0.001$). Steers in the HYD treatments had greater Cu intake compared with ORG ($P \leq 0.002$), while, ING had greater Cu intake compared to ORG and HYD ($P \leq 0.001$). However, this could be a function of ING having greater dry matter intake than ORG ($P = 0.04$).

There was a treatment × time effect on plasma Cu concentrations ($P < 0.001$). Although steers started with similar d 0 plasma Cu concentrations (average = 0.82 mg/L; $P = 0.20$), by d 28 CON steers had lesser plasma Cu concentrations compared with all other treatments ($P \leq 0.001$), which progressively decreased across the trial ($P \leq 0.001$). Steers receiving supplemental Cu were able to maintain plasma Cu concentrations above CON throughout the trial. On d 85, steers receiving 5 ppm supplemental Cu from ORG or HYD had decreased plasma Cu concentrations compared to d 28 ($P \leq 0.007$). Steers receiving 5 ppm of supplemental Cu from ING or those receiving 10 ppm from ING or ORG had similar plasma Cu concentrations throughout the trial ($P \geq 0.23$).

As plasma concentrations fell rapidly in steers receiving no supplemental Cu, it is suggested that the homeostatic mechanism controlling plasma Cu concentrations was overwhelmed. Liver Cu stores can be released to maintain plasma Cu concentrations until liver Cu stores fall below 30 ppm, and final liver Cu concentrations were below 30 ppm in steers receiving 0 or 5 ppm supplemental Cu. However, steers receiving 10 ppm supplemental Cu were able to maintain final liver Cu concentrations above 30 ppm, thus maintaining plasma Cu status.

Relative bioavailability tended ($P = 0.07$) to be increased in HYD (112%) vs. ING (set artificially at 100%), but was not different between HYD and ORG ($P = 0.22$) or ORG vs. ING ($P = 0.65$), when determined by liver Cu concentrations. Relative bioavailability of HYD and ORG were not different from one another ($P = 0.97$) or from ING ($P \geq 0.91$) when determined from plasma Cu concentrations. Previous studies have shown greater differences in relative bioavailability in organic and hydroxy sources compared to $CuSO_4$, than those in the present study. Possible factors that could be affecting relative bioavailability calculations include the basal Cu concentration of the test diet, and initial liver Cu concentrations of the animals in the trial. Additionally, rumen pH as affected by experimental diet type could also influence relative bioavailability studies; as rumen pH would be expected to be greater in a high-forage diet compared to a high-concentrate based diet. These factors should be considered when interpreting results of trace mineral bioavailability studies.

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Table 1. Diet composition

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, ppm	4.5
Mo, ppm	6.8

[1]Supplemental Cu premix provided 5% diet DM as distillers grains and contributed 0, 5, or 10 ppm supplemental Cu of complete diet from either $CuSO_4$

[2]Basal 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 a % of total diet DM: dried distillers grains 2.85%, limestone 0.5%, vitamin A and E premix 0.11%m calcium sulfate 0.9%, salt 0.31%, urea 0.3%, Rumensin 90 0.008%.

[3]DM and Cu were determined by Hansen laboratory, an all other values were determined by Dairyland laboratories

[4]DM 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 May 30, 2017.

Table 2. Influence of supplemental Cu concentration and source on liver Cu concentrations of steers

	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
DMI, lbs/d	19.4	21.7	19.5	20.7	21.7	20.4	20.8	0.767	0.17	0.10	0.63	0.35	0.23	0.04

[1]Treatment: CON = common TMR with no supplemental Cu, ING5 = common TMR plus 5 mg Cu/kg DM from $CuSO_4$, 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 $CuSO_4$, 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.

[2]Contrast: 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.

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

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

[5]Total Cu intake per steer over the entire trial.

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 × time interaction ($P \leq 0.001$).