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Bioavailability of copper from tribasic copper chloride and copper sulfate in growing cattle

J.W. Spears*, E.B. Kegley[1], L.A. Mullis

Department of Animal Science and Interdepartmental Nutrition Program, North Carolina State University, Campus Box 7621, Raleigh, NC 27695-7621, USA

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Abstract

Two experiments were conducted to determine the bioavailability of copper (Cu) from tribasic Cu chloride ($Cu_2(OH)_3Cl$) relative to Cu sulfate in growing steers. Experiment 1 compared tribasic Cu chloride to sulfate in terms of ability to maintain Cu status when supplemented to steers fed diets high in the Cu antagonists, molybdenum (Mo) and sulfur (S). Sixty Angus and Angus × Hereford steers (257 ± 2 kg body weight) were stratified by body weight and randomly assigned to treatments. Treatments consisted of 0, 5 or 10 mg supplemental Cu/kg diet DM from either Cu chloride or Cu sulfate. All diets were supplemented with 5 mg Mo/kg and 1.5 g S/kg. The control corn silage based diet analyzed 4.9 mg Cu/kg and 6.9 mg Mo/kg and was calculated to contain 3.0 g S/kg. Plasma and liver Cu concentrations and plasma ceruloplasmin activity decreased ($P < 0.01$) in all treatment groups during the 98-day study. Copper supplemented steers had higher ($P < 0.01$) plasma Cu, plasma ceruloplasmin, and liver Cu than controls at the end of the study. Steers supplemented with 10 mg Cu/kg had higher ($P < 0.01$) plasma Cu, plasma ceruloplasmin, and liver Cu than those receiving 5 mg Cu/kg diet. Bioavailability of Cu from Cu chloride, relative to Cu sulfate, was estimated from plasma Cu and ceruloplasmin on day 84 and liver Cu on day 98 using multiple linear regression and a slope ratio technique. Compared with Cu sulfate (1.00), relative bioavailability of Cu from tribasic Cu chloride was 1.32 ($P < 0.08$), 1.18 ($P < 0.38$) and 1.96 ($P < 0.04$) based on plasma Cu, plasma ceruloplasmin and liver Cu, respectively. In experiment 2, 43 Angus and Simmental steers (375 ± 7 kg BW) that had previously been depleted of Cu were used in a 21-day repletion study. Steers were randomly assigned within breed to treatment, and individually fed a corn silage based diet low in Mo (1.18 mg/kg). Treatments consisted of 0, 50 or 100 mg supplemental Cu/day from either Cu chloride or Cu sulfate. Plasma Cu, plasma ceruloplasmin and liver Cu increased ($P < 0.01$) in Cu supplemented, but not in control, steers. Plasma and liver Cu

Abbreviations: AA, amino acid; BW, body weight; Cu, copper; $CuSO_4 \cdot 5H_2O$, copper sulfate pentahydrate; CP, crude protein; Mo, molybdenum; S, sulfur; $Cu_2(OH)_3Cl$, tribasic copper chloride

* Corresponding author. Tel.: +1-919-515-4008; fax: +1-919-515-4463.
E-mail address: jerry_spears@ncsu.edu (J.W. Spears).
[1] Present address: Department of Animal Science, University of Arkansas, Fayetteville, AR 72701, USA.

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concentrations increased ($P < 0.01$) to a greater extent in steers receiving 100 mg Cu/day compared to those given 50 mg Cu/day. Tribasic Cu chloride and Cu sulfate were similar ($P > 0.10$) in their ability to increase Cu status in Cu depleted steers fed a diet low in Mo. Tribasic Cu chloride is more bioavailable than $CuSO_4$ when added to diets high in the Cu antagonists Mo and S. When evaluated in Cu depleted steers fed diets low in Mo, the two Cu sources had a similar bioavailability.

Keywords: Copper; Steers; Bioavailability

1. Introduction

Copper deficiency is a major problem in cattle in many areas of the world (Underwood and Suttle, 1999), primarily due to low bioavailability of Cu. Antagonists, such as molybdenum (Mo), sulfur (S) and iron, are frequently high in ruminant diets and reduce the bioavailability of Cu (Underwood and Suttle, 1999). Sulfur and Mo interact in the rumen to form thiomolybdates that form insoluble complexes with Cu that are poorly absorbed (Suttle, 1991).

A form of Cu that would not interact with Mo and S in the rumen, and thus, remain available for absorption, would be desirable. Tribasic Cu chloride has a very low water solubility relative to Cu sulfate (Miles et al., 1998), and may be more resistant to interactions with Mo and S in the rumen. This could result in Cu from tribasic Cu chloride being more available for absorption following solubilization in the acid environment of the abomasum. In chicks, tribasic Cu chloride was similar in bioavailability to Cu sulfate (Miles et al., 1998). Tribasic Cu chloride was also as effective as Cu sulfate in stimulating growth of weanling pigs when fed at pharmacological concentrations (Cromwell et al., 1998).

The present studies were conducted to determine the bioavailability of Cu from tribasic Cu chloride relative to Cu sulfate in steers fed diets high in Mo and S, and in Cu depleted steers fed diets low in Mo.

2. Materials and methods

2.1. Solubility of copper sources

In vitro incubations were performed in deionized water and acid to determine the solubility of Cu from Cu sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$; Eastern Minerals Corp., Henderson, NC, USA) and tribasic Cu chloride ($Cu_2(OH)_3Cl$; Micronutrients Inc., Indianapolis, IN, USA). Feed grade sources of Cu were used in all experiments. Copper sources (0.05 g Cu) were brought to a final volume of 100 ml in deionized water or 0.1% HCl (pH 2.22). Samples in water were incubated for 24 h, while those in acid were incubated for either 1 or 3 h. Subsequent incubations were conducted to determine effects of pH and amino acid (AA) ligands on solubility of Cu from tribasic Cu chloride. Samples were incubated at a pH of 2, 3, 4, 4.5 or 5 with and without the addition of glycine and lysine. Glycine and lysine were added to provide two moles of each AA per mole of Cu. All incubations were completed

at 39 °C in a shaking water bath. After incubation, samples were filtered through Whatman 541 ashless filter paper, and filtrates were analyzed for Cu by flame atomic absorption spectrophotometry (Model 5000, Perkin-Elmer, Norwalk, CT, USA). Samples were analyzed in duplicate for each solvent, pH, and incubation time.

2.2. Experiment 1

This study was conducted to compare tribasic Cu chloride and Cu sulfate in terms of their ability to maintain Cu status in steers fed diets high in Mo and S. Handling and sampling of animals in this and the subsequent experiment were approved by the North Carolina State University Animal Care and Use Committee.

Sixty Angus and Angus × Hereford steers (initially 257 ± 2 kg) were stratified by weight and randomly assigned to treatments. Treatments consisted of: (1) control (no supplemental Cu), (2) 5 mg Cu/kg diet DM from Cu sulfate, (3) 10 mg Cu/kg diet from Cu sulfate, (4) 5 mg Cu/kg diet from Cu chloride, and (5) 10 mg Cu/kg diet from Cu chloride. All experimental diets were supplemented with 5 mg Mo/kg, from sodium molybdate, and 0.15% S, from calcium sulfate. Steers were offered ad libitum a corn silage based diet (Table 1) formulated to meet nutrient requirements (NRC, 1996) with the exception of Cu. The control diet analyzed (DM basis) 11.2% crude protein (CP), 4.9 mg Cu/kg and 6.9 mg Mo/kg, and was calculated to contain approximately 3.0 g S/kg.

Twenty steers were assigned to the control treatment and 10 steers were assigned to each of the Cu supplemented treatments. Steers were housed in groups of five in covered, slotted floor pens. The experiment lasted 98 days. Liver biopsy samples were obtained on days 0 and 98 of the study, as described by Erwin et al. (1956). Jugular blood samples were collected in heparinized tubes on days 0, 28, 56 and 84 for determination of plasma Cu concentration and ceruloplasmin activity (a Cu metalloenzyme). Steers were weighed on two consecutive days at the initiation and termination of the study. Interim body weights (BW) were taken at 28-day intervals and feed intake was measured daily.

Table 1
Ingredient composition (g/kg DM) of experimental diets

	Experiment 1	Experiment 2
Corn silage	900	900
Soybean meal (48% CP, solvent)	70	46
Corn grain (ground)	14.2	36.9
Urea	5.0	6.5
Sodium chloride	2.5	2.0
Calcium sulfate	8.0	8.3
Vitamin premix[a]	0.2	0.2
Trace mineral premix[b]	0.1	0.1

[a] Vitamin premix contained per kg: 6,608,000 IU of Vitamin A, 3,524,000 IU of Vitamin D, and 6608 IU of Vitamin E.

[b] Supplied in diet (mg/kg): Zn, 30 (as $ZnSO_4$); Mn, 20 (as $MnSO_4$); I, 0.5 (as $Ca(IO_3)_2$); Co, 0.1 (as $CoCO_3$); Se, 0.1 (as Na_2SeO_3).

Data were statistically analyzed by least squares analysis of variance for a completely randomized design using the GLM procedure of SAS (1988). Liver and plasma data were analyzed as repeated measures with a model containing treatment, time, and treatment × time interaction. When a treatment × time interaction was observed, data were analyzed by sampling day, and day 0 values were used as a covariant. Differences among treatments were determined using the following single degree of freedom contrasts: (1) control versus Cu supplemented treatments, (2) 5 mg Cu/kg versus 10 mg Cu/kg, (3) Cu sulfate versus Cu chloride treatments, and (4) 10 mg Cu/kg from Cu sulfate versus 10 mg Cu/kg from Cu chloride. Relative Cu bioavailability from tribasic Cu chloride was determined, using Cu sulfate as the standard source, by multiple linear regression and a slope ratio method (Littell et al., 1995). Plasma Cu and ceruloplasmin on day 84 and liver Cu on day 98 were used in the multiple linear regression analysis.

2.3. Experiment 2

This study was conducted to compare the bioavailability of Cu from tribasic Cu chloride and Cu sulfate when supplemented to Cu depleted steers. Twenty-three Angus and 20 Simmental steers that had previously been depleted of Cu (Mullis et al., 2003a), by feeding high dietary iron, were used in a 21-day repletion study. Steers had an average initial weight of 375 ± 7 kg, and were randomly assigned within breed to treatments; which consisted of: (1) control (no supplemental Cu), (2) 50 mg Cu/day from Cu sulfate, (3) 100 mg Cu/day from Cu sulfate, (4) 50 mg Cu/day from Cu chloride, and (5) 100 mg Cu/day from Cu chloride. There were nine steers in each of the control and 50 mg Cu treatments and eight steers in each of the 100 mg Cu treatments. Steers were housed in covered pens with slotted floors and fed individually using electronic feeders (American Calan, Northwood, NH, USA). A corn silage based diet (Table 1) was offered ad libitum. The basal diet analyzed (DM basis) 10.5% CP, 4.7 mg Cu/kg and 1.18 mg Mo/kg. Before daily feeding of the silage based diet, each steer was fed 0.45 kg of ground corn grain, which served as the carrier of the appropriate Cu treatment.

The BWs were obtained prior to feeding at the beginning and end of the 21-day study. Blood samples were obtained via jugular venipuncture for plasma Cu and ceruloplasmin activity on days 0, 7, 14, and 21. A liver biopsy was obtained for Cu determination on days 0 and 21.

Data were statistically analyzed by least squares analysis of variance using the GLM procedure of SAS (1988). Plasma and liver data were analyzed as repeated measures with a model containing treatment, breed, time, treatment × breed, time × treatment, time × breed, and time × treatment × breed. When a treatment × time interaction was observed, data were analyzed by sampling day, and day 0 values were used as a covariant. Differences among treatments were determined using the following single degree of freedom contrasts: (1) control versus Cu supplemented treatments, (2) 50 versus 100 mg Cu/day, (3) Cu sulfate versus Cu chloride, and (4) 100 mg Cu from Cu sulfate versus 100 mg Cu from Cu chloride. Day 21 liver and plasma data were used to estimate relative Cu bioavailability from Cu chloride using multiple linear regression and a slope ratio method (Littell et al., 1995). A $\log_{10}$ transformation of liver and plasma data was made to account for heterogeneity of variances.

2.4. Analytical procedures

Copper in plasma, feed and liver was determined by atomic absorption spectroscopy (Model 5000, Perkin-Elmer, Norwalk, CT, USA). Standards for plasma Cu were prepared in 10% glycerin. Feed and liver samples were prepared for Cu analysis by wet ashing using nitric acid and hydrogen peroxide in a microwave digester (Model MDS-81D, CEM, Matthews, NC, USA) as described by Ward et al. (1996). Plasma ceruloplasmin activity was determined by the procedure of Houchin (1958), and activity is expressed as absorbance units at 525 nm. The CP was determined by a Kjeldahl procedure using a Kjeltec Auto 1030 Analyzer (Tecator, Hoganas, Sweden).

3. Results

3.1. Solubility of copper sources

Copper from Cu sulfate was soluble in water and 0.1% HCl (Table 2). Tribasic Cu chloride was almost totally insoluble in water. However, 767 and 868 g/kg of Cu from Cu chloride was soluble after incubation in 0.1% HCl for 1 and 3 h, respectively.

Incubations also were conducted to determine the solubility of Cu from Cu chloride at different pH with or without the presence of AA that can chelate Cu. In the absence of AA, Cu chloride was relatively insoluble at a pH of 3.0 or higher (Table 3). The addition of glycine and lysine to the HCl solution greatly increased the amount of Cu soluble, especially at pH of 3.0 or higher. Even at a pH of 4.0–5.0 approximately 330 g/kg of Cu from Cu chloride was soluble following incubation for 1 h with glycine and lysine.

3.2. Experiment 1

Plasma Cu was affected by time ($P < 0.01$) and there was a treatment × time interaction ($P < 0.01$). Copper concentrations in plasma decreased with time in all treatment groups (Table 4). Control steers had lower ($P < 0.05$) plasma Cu concentrations by day 28 and at subsequent sampling days versus Cu supplemented steers. Steers supplemented with 10 mg Cu/kg had higher ($P < 0.05$) plasma Cu concentrations than those supplemented with 5 mg Cu/kg on days 56 and 84. Plasma Cu tended to be higher in steers supplemented with 10 mg

Table 2
Solubility of copper from different sources following incubation in water or dilute acid (pH 2.22)

Diluent	Incubation time (h)	Copper source	
		Sulfate (g/kg)	Chloride (g/kg)
Water	24	945	6
HCl (0.1%)	1	983	767
	3	968	868

Table 3
Effect of pH and amino acids on solubility of copper from tribasic copper chloride

pH	g/kg
2.0	791
2.0 + glycine and lysine[a]	986
3.0	88
3.0 + glycine and lysine[a]	569
4.0	11
4.0 + glycine and lysine[a]	334
4.5	4
4.5 + glycine and lysine[a]	348
5.0	2
5.0 + glycine and lysine[a]	326

[a] Glycine and lysine were added to provide two moles of each amino acid per mole of copper.

Cu/kg from Cu chloride than in those supplemented with 10 mg Cu/kg from Cu sulfate on days 56 ($P < 0.20$) and 84 ($P < 0.08$).

Ceruloplasmin activity in plasma also decreased ($P < 0.01$) with time, especially in control steers (Table 4). Copper supplemented steers had higher ceruloplasmin activities versus controls on days 28 ($P < 0.08$), 56 ($P < 0.01$), and 84 ($P < 0.01$). Plasma ceruloplasmin activity was higher ($P < 0.05$) in steers supplemented with 10, compared to those receiving 5 mg Cu/kg diet on days 56 and 84. Copper source did not affect ceruloplasmin activity.

Liver Cu concentrations were affected by time ($P < 0.01$) and there was a treatment × time interaction ($P < 0.08$). Initial liver Cu concentration was not affected by treatment (Table 4). Means for day 98 liver Cu concentrations were adjusted using initial liver Cu as a covariant. Compared to day 0 values, liver Cu decreased ($P < 0.01$) greatly in all treatment groups by day 98. However, liver Cu concentrations were lower ($P < 0.01$) in controls versus Cu supplemented steers, and in steers supplemented with 5 mg, compared to those fed the 10 mg Cu/kg diet. Liver Cu was not affected by Cu source.

Bioavailability of tribasic Cu chloride relative to Cu sulfate was estimated from plasma Cu and ceruloplasmin values on day 84, and from liver Cu concentration on day 98 using multiple linear regression and a slope ration method. Slopes and relative bioavailability estimates are in Table 5. Compared with Cu sulfate (1.00), relative bioavailability of Cu from tribasic Cu chloride was 1.32 ($P < 0.08$), 1.18 ($P < 0.38$) and 1.96 ($P < 0.04$) using slope ratios for plasma Cu, plasma ceruloplasmin, and liver Cu, respectively.

Addition of either 5 or 10 mg Cu/kg to the control diet increased gain ($P < 0.01$) and gain:feed ($P < 0.06$) during the 98-day study (Table 6), despite the decline in plasma and liver Cu concentrations with time observed even in Cu supplemented steers. Feed intake was not affected ($P < 0.14$) by Cu supplementation.

3.3. Experiment 2

All measures of Cu status were affected by a treatment × time interaction ($P < 0.01$). During the 21 days study, plasma and liver Cu concentrations, and plasma ceruloplasmin

Table 4
Effect of copper level and source on plasma and liver copper concentrations, and ceruloplasmin activities in steers fed diets high in Mo and S (experiment 1)

	Treatment					S.E.	Contrasts (*P*-value)			
	Control	Cu sulfate (5 mg/kg)	Cu sulfate (10 mg/kg)	Cu chloride (5 mg/kg)	Cu chloride (10 mg/kg)		Control vs. Cu	5 vs. 10 mg Cu/kg	Cu sulfate vs. Cu chloride	10 mg/kg Cu sulfate vs. 10 mg/kg Cu chloride
Plasma Cu (mg/l)										
Day 0	0.91	1.00	0.91	0.96	1.02	0.05	0.22	0.78	0.52	0.17
Day 28	0.71	0.83	0.80	0.73	0.86	0.05	0.04	0.33	0.66	0.45
Day 56	0.43	0.66	0.71	0.62	0.83	0.06	0.01	0.04	0.57	0.19
Day 84	0.29	0.51	0.65	0.50	0.79	0.04	0.01	0.01	0.21	0.07
Plasma ceruloplasmin[a]										
Day 0	0.161	0.180	0.167	0.170	0.181	0.011	0.23	0.95	0.88	0.43
Day 28	0.126	0.153	0.149	0.126	0.160	0.012	0.07	0.23	0.53	0.54
Day 56	0.037	0.072	0.084	0.056	0.100	0.010	0.01	0.01	0.99	0.28
Day 84	0.011	0.034	0.059	0.029	0.071	0.007	0.01	0.01	0.67	0.28
Liver Cu (mg/kg DM)										
Day 0	83.8	97.8	82.3	83.9	114.7	15.5	0.45	0.64	0.57	0.16
Day 98[b]	12.2	13.0	31.5	16.1	38.3	4.2	0.01	0.01	0.29	0.31

[a] Expressed as absorbance units.
[b] Day 0 liver Cu was used as a covariant.

Table 5
Estimated relative bioavailability of copper sources in steers fed diets high in Mo and S, based on multiple linear regression of plasma Cu, plasma ceruloplasmin, and liver Cu on dietary Cu concentration (experiment 1)

Dependent variable	Cu source	Slope ± S.E.	*P*-value[a]	Relative bioavailability
Plasma Cu[b]	Sulfate	0.037 ± 0.006	0.07	1.00
	Chloride	0.049 ± 0.006		1.32
Plasma ceruloplasmin[b]	Sulfate	0.0050 ± 0.0009	0.38	1.00
	Chloride	0.0059 ± 0.0009		1.18
Liver Cu[c]	Sulfate	2.17 ± 0.91	0.03	1.00
	Chloride	4.25 ± 0.91		1.96

[a] *P*-value for slope differences among Cu sources.
[b] Day 84 values were used in regression analysis.
[c] Day 98 values were used in regression analysis.

did not change greatly from day 0 values in steers fed the non-Cu supplemented control diet (Table 7). Addition of 50 or 100 mg Cu/day increased ($P < 0.01$) plasma Cu concentration and ceruloplasmin activity on days 7, 14, and 21. Steers receiving the higher dose of Cu had higher ($P < 0.01$) plasma Cu than those supplemented with 50 mg Cu/day on days 7, 14, and 21. Compared to those receiving 50 mg Cu/day, steers supplemented with 100 mg Cu/day had higher ceruloplasmin activity on days 14 ($P < 0.05$) and 21 ($P < 0.08$).

Liver Cu on day 21 was increased ($P < 0.01$) by supplemental Cu, and steers supplemented with 100 mg Cu/day had higher ($P < 0.01$) liver Cu than those receiving 50 mg (Table 7). Although plasma Cu and ceruloplasmin increased in steers fed 50 mg Cu/day, this level of supplementation did not increase liver Cu stores to any extent during the 21-day repletion period.

All indices of Cu status (plasma Cu, plasma ceruloplasmin, and liver Cu) were lower ($P < 0.05$) in Simmental versus Angus steers at all sampling dates (data not shown). However, no treatment × breed interactions were observed.

No differences were observed between tribasic Cu chloride and Cu sulfate in regard to ability to increase Cu status in Cu depleted steers fed diets low in Mo. Multiple linear regression of plasma and liver Cu, and plasma ceruloplasmin activity on day 21 on Cu intake also indicated that the Cu sources had similar bioavailabilities (Table 8).

4. Discussion

4.1. Solubility of copper sources

Results of the present study suggest that tribasic Cu chloride would be rather insoluble in the rumen environment but at least partially soluble in the acid environment of the abomasum. Miles et al. (1998) found that Cu chloride was insoluble in water but completely soluble following incubation in 0.4% HCl for 1 h. The pH in the abomasum can be higher (Knight et al., 1972) than the 2.2 pH evaluated in the present study. Ligands or chelators present in abomasal digesta also may enhance the solubility of Cu from Cu chloride. Therefore,

Table 6
Effect of copper level and source on performance of steers fed diets high in Mo and S (experiment 1)

	Treatment					S.E.	Contrasts (*P*-value)			
	Control	Cu sulfate (5 mg/kg)	Cu sulfate (10 mg/kg)	Cu chloride (5 mg/kg)	Cu chloride (10 mg/kg)		Control vs. Cu	5 vs. 10 mg Cu/kg	Cu sulfate vs. Cu chloride	10 mg/kg Cu sulfate vs. 10 mg/kg Cu chloride
Daily gain (kg/day)	0.93	0.99	1.10	1.08	1.12	0.05	0.01	0.20	0.29	0.77
DM intake (kg/day)	7.82	8.12	8.59	8.41	8.35	0.36	0.14	0.61	0.96	0.66
Gain:feed	0.119	0.122	0.128	0.129	0.134	0.005	0.06	0.28	0.22	0.40

Table 7
Effect of copper level and source on plasma and liver copper concentrations, and ceruloplasmin activities in steers (experiment 2)

	Treatment					S.E.	Contrasts (*P*-value)			
	Control	Cu sulfate (50 mg/day)	Cu sulfate (100 mg/day)	Cu chloride (50 mg/day)	Cu chloride (100 mg/day)		Control vs. Cu	50 vs. 100 mg Cu/day	Cu sulfate vs. Cu chloride	100 mg Cu/day Cu sulfate vs. 100 mg Cu/day Cu chloride
Plasma Cu (mg/l)										
Day 0	0.20	0.27	0.22	0.25	0.24	0.04	0.30	0.51	0.99	0.78
Day 7	0.28	0.45	0.55	0.48	0.55	0.03	0.01	0.01	0.54	0.67
Day 14	0.30	0.57	0.78	0.62	0.74	0.04	0.01	0.01	0.88	0.50
Day 21	0.34	0.69	0.83	0.70	0.83	0.04	0.01	0.01	0.88	0.99
Plasma ceruloplasmin[a]										
Day 0	0.084	0.080	0.079	0.083	0.076	0.010	0.75	0.60	0.91	0.82
Day 7	0.070	0.105	0.115	0.108	0.113	0.010	0.01	0.45	0.98	0.88
Day 14	0.075	0.125	0.153	0.126	0.145	0.010	0.01	0.03	0.77	0.64
Day 21	0.057	0.130	0.141	0.127	0.153	0.010	0.01	0.07	0.64	0.40
Liver Cu (mg/kg DM)[b]										
Day 0	9.7	13.6	10.1	14.6	11.3	2.1	0.22	0.13	0.61	0.70
Day 21[b]	10.1	14.2	30.1	15.8	25.5	2.9	0.01	0.01	0.61	0.28

[a] Expressed as absorbance units.
[b] Day 0 liver Cu was used as a covariant.

Table 8
Estimated relative bioavailability of Cu sources when fed to Cu depleted steers (experiment 2)

Dependent variable	Cu source	Slope ± S.E.	*P*-value[a]	Relative bioavailability
Plasma Cu[b]	Sulfate	0.0040 ± 0.0006	0.76	1.00
	Chloride	0.0041 ± 0.0007		1.04
Plasma ceruloplasmin[b]	Sulfate	0.0037 ± 0.0008	0.61	1.00
	Chloride	0.0041 ± 0.0008		1.10
Liver Cu[b]	Sulfate	0.0049 ± 0.0007	0.35	1.00
	Chloride	0.0043 ± 0.0007		0.87

[a] *P*-value for slope differences among Cu sources.
[b] Day 21 values were used in regression analysis.

incubations were conducted to determine the effect of pH and the presence of AA, that can chelate Cu, on solubility of Cu from Cu chloride. Addition of glycine and lysine to the incubation solution, to provide two moles of each AA per mole of Cu, greatly increased solubility of Cu from Cu chloride, particularly within the pH range of 3.0–5.0. The increased solubility of Cu in the presence of AA may be due to the AA complexing Cu, and thus preventing an increase in pH associated with Cu becoming soluble.

4.2. Experiment 1

The present study suggest that tribasic Cu chloride is a more bioavailable source of Cu than Cu sulfate, when supplemented to cattle diets high in the Cu antagonists, Mo and S. The higher bioavailability of Cu from tribasic Cu observed in steers fed high dietary Mo and S may relate to the lower solubility of Cu chloride observed at neutral or slightly acidic pH. Thiomolybdates, that are formed in the rumen, when both dietary Mo and S are high, result in a high portion of ruminal Cu existing in an insoluble form, associated with high molecular weight proteins (Allen and Gawthorne, 1987). If Cu chloride is insoluble in the rumen, this may prevent the Cu from forming stable complexes, and thus result in a greater quantity of the supplemental Cu being available for absorption in the small intestine. Copper sulfate is readily soluble in water. However, interactions that occur result in most of the Cu from Cu sulfate being in an insoluble form in the rumen, especially when dietary Mo is high (Ivan and Veira, 1985).

Steers had plasma and liver Cu concentrations within the normal range (Underwood and Suttle, 1999) on day 0. However, by the end of the 98-day study plasma and liver Cu concentrations in control steers had decreased to concentrations consistent with a marginal Cu deficiency (Underwood and Suttle, 1999). A number of studies (Humphries et al., 1983; Ward et al., 1996) have demonstrated that Mo addition to diets low in Cu greatly decreases plasma and liver Cu concentrations in cattle. Copper supplementation (5 or 10 mg/kg) of the control diet, high in Mo and S, increased gain and gain:feed in the present study. Previous studies (Humphries et al., 1983; Phillippo et al., 1987; Gengelbach et al., 1994) have shown that dietary supplementation of Mo, at concentrations similar to those used in the present study, reduces gain and efficiency of gain in cattle fed diets low in Cu.

4.3. Experiment 2

Underwood and Suttle (1999) suggested that liver Cu concentrations less than 19 mg/kg DM or serum Cu concentrations less than 0.58 mg/l may indicate marginal Cu deficiency in ruminants. Based on these criteria, steers used in the Cu repletion study were at least marginally deficient in Cu at the beginning of the study. Copper chloride and Cu sulfate increased plasma and liver Cu concentrations and plasma ceruloplasmin activity to a similar extent when supplemented to Cu depleted steers for 21 day. In contrast to the high dietary Mo used in experiment 1, diets used in the Cu repletion study were low in Mo (1.18 mg/kg). This would result in a greater bioavailability of Cu in experiment 2 (Underwood and Suttle, 1999).

The lower plasma and liver Cu concentrations and plasma ceruloplasmin activity in Simmental versus Angus steers was expected. When fed diets low or marginal in Cu, Simmental cattle have been shown to have lower Cu status than Angus (Ward et al., 1995; Mullis et al., 2003a,b).

5. Conclusions

When evaluated in Cu depleted steers fed diets low in Mo, tribasic Cu chloride had similar bioavailability to Cu sulfate. However, Cu from tribasic Cu chloride appeared to be more bioavailable than Cu sulfate when supplemented to diets high in Mo and S. Because of its low solubility at neutral or slightly acidic pH, tribasic Cu chloride may interact with Mo and S to a lesser extent than Cu sulfate in the rumen, and this could explain the higher Cu bioavailability.

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