Effect of Copper Source and Level on the Rate and Extent of Copper Repletion in Holstein Heifers¹

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ABSTRACT

The objective of this study was to evaluate the rate and extent of Cu repletion in Holstein heifers using two Cu sources (organic and inorganic) at two levels (15 and 30 mg/kg). An additional repletion treatment included a Cu oxide bolus. Heifers ($n = 50$) were individually fed a total mixed ration fortified with S and Mo at 0.40%, and 15 mg/kg of dry matter of the total diet, respectively. After 111 d of depletion, heifers were stratified by liver Cu concentration and randomly allotted to one of five repletion treatments. Four treatments consisted of feed sources of Cu (feed-Cu), 1) CuSO_4 at 15 mg/kg; 2) CuSO_4 at 30 mg/kg; 3) Availa-Cu at 15 mg/kg; and 4) Availa-Cu at 30 mg/kg. Availa-Cu is an organic Cu source that produces a Cu-amino acid complex. A fifth treatment, consisting of an intraruminal bolus (IB), provided a single dose of 25 g of CuO needles. Repletion treatments were delivered in the same total mixed ration without supplemental S and Mo. Copper status was assessed in blood and liver samples collected on 14-d intervals for 70 d. Irrespective of treatment, all heifers increased in body weight during the repletion period. Liver Cu increased in each feed-Cu treatment over time. Heifers treated with an IB reached a peak in liver Cu concentration (165.5 mg/kg) on d 28. Mean liver Cu concentrations were higher in heifers receiving 30 mg/kg of Cu compared with heifers receiving 15 mg/kg of Cu. Red blood cell superoxide dismutase (SOD) activity was higher ($P < 0.001$) in heifers receiving CuSO_4 than Availa-Cu (0.98 vs 0.87 U). Also, SOD activity was higher when

heifers were supplemented with 30 vs 15 mg/kg Cu (0.98 vs 0.87 U). Heifers receiving the Cu IB had higher SOD activity than heifers receiving feed-Cu sources (1.03 vs 0.92 U). Plasma ceruloplasmin concentration was higher ($P < 0.001$) in IB-treated heifers vs. other treatments. No differences in plasma ceruloplasmin were detected for feed-Cu source or level. These results indicate that all Cu sources evaluated in this study elevated Cu status of depleted heifers, particularly when provided at higher dietary levels. (**Key words:** copper, heifer, repletion)

Abbreviation key: ADG = average daily gain, **Feed-Cu** = Cu-supplemented diets without antagonists, **IB** = intraruminal bolus, **SOD** = Cu, Zn-superoxide dismutase.

INTRODUCTION

In the past, ruminant Cu deficiencies have usually been corrected by supplementation with inorganic mineral supplements. Trace minerals complexed with organic molecules have been implied to be more bioavailable than inorganic trace minerals (Brown and Zeringue, 1994). However, a shortcoming of supplemental organic Cu compounds is their higher cost (Attaelmannan and Reid, 1996). Some researchers (Nockels et al., 1993; Rabiansky et al., 1999) have indicated that Cu lysine may be more beneficial than CuSO_4 in correcting Cu deficiency in cattle. The physiological advantage afforded by organic Cu compounds may be due to the unique coordination chemistry of Cu, which permits the formation of highly soluble, chemically stable products that resist digestion and interaction with antagonists in the gut (Brown and Zeringue, 1994).

Other studies (Ward and Spears, 1991; Ward et al., 1993) have shown that Cu availability from Cu lysine and CuSO_4 were comparable in cattle. Similarly, Kegley and Spears (1994) reported that the Cu status of

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calves fed CuSO_4 did not differ from calves fed Cu lysine; however, feed-grade CuO powder did not improve the status of Cu deficient calves.

An intraruminal bolus (**IB**) containing CuO needles is another method that provides long-term supplementation through the slow release of Cu over an extended period. Cameron et al. (1989) reported significant increases in liver Cu concentration after CuO bolus administration in cattle consuming high-Mo forage and high- SO_4 water. Although administration of a CuO bolus effectively elevated the liver Cu concentration of Cu-deficient cows, Arthington et al. (1995b) reported significant reductions in average daily gain (**ADG**) and weaning weight of calves that received boluses.

The present study was designed to compare the efficacy of Availa-Cu 100 (Zinpro Corp., Eden Prairie, MN), an organic Cu source that produces a Cu-AA complex, CuSO_4 , and a CuO-containing bolus on the rate and extent of Cu repletion in Cu-deficient Holstein heifers.

MATERIALS AND METHODS

The University of Florida Animal Care and Use Committee approved the handling and sampling of animals in this experiment (#A486). Sixty-three 8- to 10-mo-old Holstein heifers from a northwest Florida dairy were received at the University of Florida Beef Research Unit. Upon arrival, heifers were weighed and vaccinated for *Clostridia* (Fortress 7, Pfizer Animal Health, Exton, PA), IBR, PI3, BVD, BRSV, *Leptospira* (Cattlemaster 4 L5, Pfizer Animal Health, Exton, PA), and *Brucella abortus* (Professional Biologicals Co., Denver, CO). Heifers had an average initial BW of 219 ± 4.8 kg. Heifers were stratified by BW and randomly assigned to one of five pens (100 m²). An adaptation period (34 d) was allowed to train the heifers to feed individually via electronic Calan gates (American Calan, Northwood, NH). All heifers were provided a basal diet (5.45 kg of DM/d) consisting of ground corn, soybean meal, cottonseed hulls, and molasses for a 111-d Cu-depletion period followed by a 70-d Cu-repletion period (Table 1). To induce a Cu deficiency, S and Mo were provided at 0.40% of the total diet, and 15 mg/kg (Cu:Mo = 1:3), as flowers of S and Na_2MoO_4 , respectively. From d 37 of the depletion period until completion of the experiment, a low-quality bermudagrass hay (4.6 mg/kg of Cu) was offered ad libitum due to persistent bloat problems among heifers. Hay consumption was low, but actual intake was not measured. Following depletion, heifers were blocked by liver Cu concentration and randomly allotted to one of five treatment groups (n = 10 heifers/

Table 1. Composition of depletion and repletion diets.

Item	Depletion diet ¹	Repletion diet ^{1,2}
		% of DM
Corn	55.0	60.5
Soybean meal	16.3	15.2
Cottonseed hulls	19.0	15.0
Molasses	7.0	7.0
Limestone	0.9	0.9
Dicalcium phosphate	0.9	0.9
Vitamin/mineral premix	0.9 ^{3,4}	0.5 ³

¹Diets fed for 111 and 70 d for Cu depletion and repletion, respectively. Diet DM = 84.8 and 84.4% for the Cu depletion and repletion diets, respectively.

²Analyzed Cu concentration for each repletion diet was 19.48, 29.38, 20.80, 40.39, and 4.81 mg/kg for CuSO_4 at 15 mg/kg, CuSO_4 at 30 mg/kg, Availa-Cu at 15 mg/kg, Availa-Cu at 30 mg/kg, and CuO bolus, respectively.

³Vitamin/mineral premix supplied 50, 0.10, 0.10, 20, and 20 mg/kg of Fe, Se, Co, Mn, and Zn, respectively. Vitamins A and D were supplied at 8480 IU/d and 1320 IU/d, respectively.

⁴Fortified with flowers of S to provide a dietary S level of 0.40% and 15 mg/kg of Mo as Na_2MoO_4 to achieve a Cu:Mo ratio of 1:3. Copper concentration of depletion diet was 5.50 mg/kg.

treatment). During repletion, heifers received Cu supplementation diets without antagonists (**Feed-Cu**): 1) 15 mg/kg of Cu from CuSO_4 ; 2) 30 mg/kg of Cu from CuSO_4 ; 3) 15 mg/kg of Cu from Availa-Cu; and 4) 30 mg/kg of Cu from Availa-Cu. Availa-Cu (Zinpro Corporation) is an organic Cu source derived from a manufacturing process that produces a Cu-AA complex in a metal:AA complex ratio of 1:1. Seventeen different free AA are available to participate in this complex. A fifth treatment provided heifers with a single 25-g dose of CuO needles via an IB (Copasure, Animax Ltd., Columbus, OH). These heifers received the basal diet without any additional supplemental copper.

Heifers were weighed on d 0, 14, 56, and 70 of Cu repletion. Liver biopsy samples were collected on d 0, 42, 70, and 111 of the depletion period, and d 0, 14, 28, 42, 56, and 70 of the repletion period using a standard procedure (Arthington et al., 1995a). Following each collection, samples were frozen and sent to Michigan State University (Animal Health Diagnostic Laboratory, Lansing) for analysis. Liver samples were digested and subsequently analyzed by inductively coupled plasma-atomic emission spectroscopy as described by Braselton et al. (1997).

Jugular blood was collected by venipuncture into heparinized evacuated tubes on d 0, 14, 28, 42, 56, and 70 of the repletion period. Blood samples were prepared and analyzed at the University of Florida Animal Nutrition Laboratory. Plasma for mineral and ceruloplasmin analyses was harvested following centrifugation at room temperature for 25 min at $2400 \times g$. After plasma removal, red blood cell lysate for Cu,

Table 2. Effect of Cu source and level on weight gain in heifers during Cu repletion.¹

Item	CuSO ₄ (mg/kg)		Availa-Cu (mg/kg)		CuO bolus	SEM
	15	30	15	30		
	kg					
BW						
Initial	334.7	336.1	326.3	327.8	343.6	2.8
Final	400.0	409.3	396.3	403.7	409.1	2.8
70-d gain	65.3	73.2	70.1	75.9	65.4	4.9
70-d ADG	0.93	1.05	1.00	1.08	0.93	0.07

¹Single-df contrasts are source (CuSO₄ vs Availa-Cu; $P = 0.48$), level (15 vs. 30 mg/kg; $P = 0.16$), and feed sources of Cu (CuSO₄ and Availa-Cu) vs a single 25-g dose of CuO by intraruminal bolus ($P = 0.24$).

Zn-superoxide dismutase (**SOD**) analysis was collected as described by Disilvestro and Marten (1990) on d 14, 28, 42, and 70. Lysate and plasma samples were placed in a -20°C freezer until they were analyzed. Stored plasma samples were thawed and diluted with 1:1 (vol:vol) deionized water via the method described by Miles et al. (2001). Plasma Cu concentration was determined by atomic absorption spectrophotometry (Perkin-Elmer AAS 5000, Wellesley, MA). Plasma ceruloplasmin activity was determined by spectrophotometric measurement of the colored products formed by the oxidation of *p*-phenylenediamine as described by Demetriou et al. (1974). The activity of erythrocyte SOD was determined by the method originally described by Prohaska (1983) and modified by Percival (1993). Results are expressed as units of SOD activity. One unit of SOD activity is the amount of sample required to inhibit pyrogallol autooxidation by 50%.

Data were analyzed by ANOVA using PROC GLM of SAS (1985). For all analyses involving repeated measures over time, a split-plot design was used with animal serving as the whole plot and time as the subplot. The model included the effects of treatment, time, and the time $\times$ treatment interaction. Treatment means were compared using single-degree of freedom orthogonal contrasts: CuSO₄ vs Availa-Cu (source), 15 mg/kg vs 30 mg/kg (level), and all feed-Cu sources vs. IB.

RESULTS AND DISCUSSION

Irrespective of treatment, BW increased similarly in all heifers (Table 2). Previous Cu supplementation studies with different feed sources and levels of Cu have yielded similar results. Rabiansky et al. (1999) reported no differences in total gain or ADG in Cu deficient heifers fed 8 or 16 mg/kg of Cu from CuSO₄ or Cu lysine. Engle and Spears (2000) reported no treatment effects on ADG in growing steers fed 20 or 40 mg/kg of Cu from CuSO₄ or 20 mg/kg of Cu from

either Cu citrate, Cu proteinate, or Cu chloride in a corn silage–soybean meal-based diet; however, Cu supplementation did reduce gain and feed efficiency in the same steers during the finishing phase of the experiment.

Liver Cu concentration decreased during the Cu depletion period (133, 64, and 33 mg/kg on d 0, 42, and 70 of the Cu depletion period, respectively; SEM = 2.5). The initial liver Cu concentration on d 0 of the Cu repletion period was 15 ± 5 mg/kg and was similar for all treatments. Underwood and Suttle (1999) indicated that liver Cu concentrations of 20 to 25 mg/kg or less were indicative of Cu deficiency. Liver Cu concentration increased ($P < 0.01$) over time during the repletion period (Figure 1). Liver Cu concentration for each feed-Cu treatment increased ($P < 0.01$) linearly throughout the repletion period. Similar increases in liver Cu concentration over time have been reported in cattle supplied feed sources of Cu (Arthington et al., 1995a). Stoszek et al. (1986) reported that supple-

Figure 1. Effect of Cu source and level on liver Cu concentration. Pooled SEM = 12 mg/kg. Liver Cu concentration was higher ($P < 0.05$) in heifers treated with a 25-g dose of CuO by intraruminal bolus than all heifers given feed sources of Cu on d 14 and 28. Mean liver Cu concentration over all times was higher ($P < 0.01$) in heifers fed 30 vs 15 mg/kg of Cu.

mentation of 300 to 400 mg of Cu/d to Cu-deficient cattle rapidly increased liver Cu stores to normal levels. Liver Cu concentration in heifers given a Cu IB peaked (163 ± 12 mg/kg) on d 28 and was higher ($P < 0.05$) than all feed-Cu treatments on d 14 and 28 (Figure 1). Liver Cu concentration was higher ($P < 0.01$) on d 28, 42, 56, and 70 in heifers fed 30 mg/kg of Cu from CuSO_4 than either source at 15 mg/kg of Cu, but not in heifers fed Availa-Cu at 30 mg/kg. On d 28 and all subsequent sampling dates, liver Cu concentration in heifers fed 30 mg/kg of Cu from Availa-Cu was higher ($P < 0.05$) than heifers fed 15 mg/kg of Cu from CuSO_4 . Similar to the current study, Rabiansky et al. (1999) reported that liver Cu concentration in heifers fed 16 mg/kg of Cu from CuSO_4 and Cu lysine was similar.

Organic Cu sources have been shown to have higher bioavailability. Nockels et al. (1993) reported greater apparent Cu absorption and retention of organic Cu compounds compared with CuSO_4 in calves following an 18-d stress period that included ACTH injections and mineral restriction. Du et al. (1996) reported a higher concentration of liver Cu in rats fed Cu proteinate vs. CuSO_4 . In the current study, liver Cu concentration was not affected by dietary source. Other authors (Wittenberg et al., 1990; Ward et al., 1996) have reported no differences in liver Cu concentration between inorganic and organic sources. While using Cu-depleted steers receiving 10 mg/kg of added Mo, Wittenberg et al. (1990) found that steers fed Cu proteinate had similar liver Cu concentrations compared with steers fed CuSO_4 . Ward et al. (1996) used a S and Na_2MoO_4 supplementation strategy similar to the current study to deplete heifers of Cu and found similar results; liver Cu concentration of heifers was not different when equal amounts of Cu proteinate and CuSO_4 were fed.

Feeding a Cu level of 30 mg/kg resulted in a higher ($P < 0.01$) mean liver Cu concentration compared with 15 mg/kg (Figure 1). Eckert et al. (1999) reported that liver Cu concentration in ewes decreased with higher levels of Cu proteinate, and increased with higher levels of CuSO_4 . In the current study, liver Cu concentration increased regardless of supplemental Cu source. The ewes in the study by Eckert et al. (1999) had previously received a Cu-sufficient diet without antagonists and had adequate liver Cu concentrations before receiving the Cu supplements. In contrast, heifers in the current study were initially Cu deficient.

In the current study, 20 g of Cu was delivered by the 25-g CuO bolus. A sharp increase in liver Cu concentration in IB-treated heifers had occurred by d 14, peaking by d 28. Dunbar et al. (1993) reported a peak in liver Cu concentration around 91 d. The discrepancy

in time to peak liver Cu concentration between the two studies may be due either to the difference in initial Cu status or the mass of Cu offered by the bolus. The heifers used by Dunbar et al. had adequate liver Cu concentrations before receiving 10 g of encapsulated Cu-wire particles. In the current study, the rapid increase in liver Cu concentration by d 28 may have occurred because IB-treated heifers were initially Cu deficient before bolus administration. The CuO bolus may provide a readily available form of Cu when delivered in sufficient quantities to Cu-deficient cattle. The linear increase in liver Cu concentration in heifers provided feed sources of Cu, compared with IB-treated heifers, might suggest a mechanism in which these sources of Cu are utilized differently by the animal. However, there are no specific studies that show differences in liver Cu concentration over time when feed source and level of Cu are compared with CuO boluses. In the current study, the feed-Cu sources provided a relatively small daily amount of Cu (0.08 and 0.16 g of Cu/d for heifers provided 15 and 30 mg/kg of Cu, respectively), $<1\%$ of the amount of Cu initially delivered by the CuO bolus. Stoszek et al. (1986) reported that higher supplemental levels of Cu (<400 mg of Cu/d) result in only slight additional increases in liver Cu concentration in cattle compared with lower supplemental levels. These authors indicated that once physiologically desirable liver Cu concentrations were reached, a Cu-absorption blocking or excretory mechanism might exist. Ward et al. (1996) reported that CuCO_3 was successful in maintaining plasma Cu and ceruloplasmin activity but was not as efficiently stored in the liver as CuSO_4 and an organic Cu source (Cu-proteinate). Arthington and Brown (2001) reported increases in liver Cu, along with concurrent increases in fecal Cu, 12 and 33 d after administration of a CuO bolus. The plateau in liver Cu concentration in IB-treated heifers after d 28 may be a result of homeostatic regulation of Cu absorption from CuO needles when sufficient liver Cu concentrations are obtained. The period for which IB treatment is effective in maintaining adequate Cu status in cattle is uncertain. MacPherson (1984) indicated that 20 g of CuO needles provided 11 mo of protection against Cu deficiency. Dunbar et al. (1993) reported a protection period of 12 mo. In the current study, the liver Cu concentration in IB-treated heifers was at a concentration considered adequate at the end of the repletion period on d 70.

Average plasma Cu concentration in Cu-deficient heifers before repletion was 0.29 ± 0.02 mg/kg (SEM = 0.02). Plasma Cu concentration increased ($P < 0.01$) rapidly during the first 14 d of repletion, regardless of treatment, with only slight increases after d 14

Table 3. Effect of Cu source and level on plasma Cu concentration during Cu repletion.¹

Day	CuSO ₄ (mg/kg)		Availa-Cu (mg/kg)		CuO bolus
	15	30	15	30	
	mg/kg				
0	0.26	0.27	0.32	0.32	0.27
14	0.75	0.81	0.89	0.79	0.88
28	0.84	0.79	0.88	0.84	0.85
42	0.87	0.97	0.92	0.89	0.95
56	0.79	0.80	0.82	0.85	0.88
70	0.86	0.93	0.88	0.96	0.97
Mean	0.73	0.76	0.79	0.78	0.80

¹Single-df contrasts are source (CuSO₄ vs Availa-Cu; $P = 0.24$), level (15 vs 30 mg/kg; $P = 0.72$), and feed sources of Cu (CuSO₄ and Availa-Cu) vs 25-g single dose of CuO by intraruminal bolus ($P = 0.24$). Pooled SEM = 0.04, based on 36 df.

(Table 3). After Cu depletion, all heifers had plasma and liver Cu concentrations below 0.40 and 20 mg/kg, respectively. When liver Cu concentration reached levels >40 mg/kg by d 14, plasma Cu concentration was variable. The plasma and liver Cu concentrations in this study correspond with the findings of Claypool et al. (1975). These authors indicated that when liver Cu levels are <40 mg/kg, plasma Cu levels are usually <0.5 mg/kg; however, plasma Cu levels are highly variable when liver Cu concentrations are >40 mg/kg. Plasma Cu concentration can often be an unreliable indicator of Cu deficiency, since plasma Cu concentration increases in cattle exposed to an immune challenge (Nockels et al., 1993). Therefore, plasma Cu concentration should not be the only index used to define Cu deficiency in cattle (Mulryan and Mason, 1992).

Ceruloplasmin responded in a fashion similar to plasma Cu, in that concentrations increased rapidly by d 14 ($P < 0.01$), regardless of treatment (Table 4). Similar increases in ceruloplasmin concentration (Rabiansky et al., 1999) and activity (Kegley and

Spears, 1994) over time have been reported in Cu-deficient cattle fed either CuSO₄ or Cu lysine. In the current study, plasma ceruloplasmin and plasma Cu concentrations were similar, increasing sharply by d 14. Mean plasma ceruloplasmin concentration was higher ($P < 0.05$) in IB-treated heifers than all feed-Cu treatments (22.8 and 20.6 mg/dL for IB and feed-Cu treatments, respectively; SEM = 0.84). The higher ceruloplasmin concentration in IB-treated heifers was likely due to the larger initial amount of Cu being supplied by the bolus.

Erythrocyte SOD activity increased over time ($P < 0.01$; Table 5). Heifers fed CuSO₄ had higher ($P < 0.001$) mean SOD activity than did heifers fed Availa-Cu. In addition, IB-treated heifers had higher ($P = 0.004$) mean SOD activity than all feed-Cu treatments (Table 5). We are unable to provide an explanation for these differences. Suttle and McMurray (1983) reported that a temporal relationship between plasma Cu concentration and erythrocyte SOD might exist and be of diagnostic value when evaluating Cu status.

Table 4. Effect of Cu source and level on plasma ceruloplasmin concentration during Cu repletion.¹

Day	CuSO ₄ (mg/kg)		Availa-Cu (mg/kg)		CuO bolus
	15	30	15	30	
	mg/100 ml				
0	5.9	9.1	10.1	7.6	7.9
14	19.5	20.0	21.3	18.8	23.0
28	23.7	20.3	21.7	21.2	21.6
35	24.4	25.9	24.5	22.9	27.6
42	21.5	23.1	23.5	23.1	25.0
49	21.7	23.3	23.1	24.1	25.4
56	23.3	25.0	23.4	24.6	29.2
70	27.2	25.2	27.8	27.2	30.2
Mean	20.9	21.5	21.9	21.2	23.7

¹Single-df contrasts are source (CuSO₄ vs Availa-Cu; $P = 0.40$), level (15 vs 30 mg/kg; $P = 0.93$), and feed sources of Cu (CuSO₄ and Availa-Cu) vs a 25-g single dose of CuO by intraruminal bolus ($P < 0.0001$). Pooled SEM = 0.92, based on 36 df.

Table 5. Effect of Cu source and level on erythrocyte superoxide dismutase activity during Cu repletion.¹

Day	CuSO ₄ (mg/kg)		Avala-Cu (mg/kg)		CuO bolus
	15	30	15	30	
	Units of inhibition				
14	0.74	0.83	0.63	0.76	0.85
28	0.85	0.93	0.60	0.80	0.89
42	0.95	1.02	0.84	0.97	0.92
70	1.28	1.27	1.08	1.23	1.48
Mean	0.95	1.01	0.79	0.94	1.03

¹Single-df contrasts are source (CuSO₄ vs Avala-Cu; $P < 0.001$), level (15 vs 30 mg/kg; $P < 0.002$), and feed sources of feed (CuSO₄ and Avala-Cu) vs a 25-g single dose of CuO by intraruminal bolus ($P = 0.004$). Pooled SEM = 0.08, based on 36 df.

These investigators found that erythrocyte SOD concentration reached maximal levels 15 d after plasma Cu concentration peaked in ewes supplied 0.5 g of Cu as CuSO₄ in a gelatin capsule. In the current study, plasma Cu concentration reached peak values by d 14; however, SOD activity continued to increase up to d 70. The differences in the lag time for peak recovery of plasma Cu concentration and erythrocyte SOD activity may be the result of several factors. First, the ewes in the Suttle and McMurray (1983) study may have been more severely Cu deficient than the heifers in the current study prior to repletion. Second, the larger lag time in the current study may be a result of the long erythrocyte life span (~150 d), or the incorporation of Cu into SOD at erythropoiesis. Continued sampling beyond 70 d of supplementation may have provided more insight to the interpretation of these data.

CONCLUSIONS

Both CuSO₄ and Avala-Cu appear equally effective in elevating the Cu status of Cu-deficient Holstein heifers. Copper supplementation at 30 mg/kg significantly increased liver Cu concentration compared with Cu supplementation at 15 mg/kg. In comparison to feed-Cu sources, administration of the CuO bolus (one-time dose of 20 g of Cu) resulted in a higher mean plasma ceruloplasmin concentration and a more rapid increase in liver Cu concentration, which peaked at d 28 and then plateaued.

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