

# Effects of copper supplementation on feedlot performance, carcass characteristics, and rumen sulfur metabolism of growing cattle fed diets containing 60% dried distillers grains<sup>1</sup>

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**ABSTRACT:** The effects of 3 supplemental Cu concentrations on feedlot performance, mineral absorption, carcass characteristics, and ruminal S metabolism of cattle fed diets containing 60% dried distillers grains with solubles (DDGS) were evaluated in 2 experiments. Experiment 1 was conducted with 84 Angus-cross yearling steers and heifers (initial BW = 238 ± 36 kg), which were blocked by gender and allocated to 12 pens. Supplemental dietary Cu (tribasic copper chloride) treatments were: 1) 0 mg Cu/kg diet DM, 2) 100 mg Cu/kg diet DM, 3) 200 mg Cu/kg diet DM. The remainder of the diet was DDGS (60%), grass hay (10%), pelleted soy hulls (15%), and a vitamin-mineral supplement (15%). Diets were offered ad libitum throughout the finishing phase (168 d). Three cattle from each pen (n = 36) were harvested on d 168 and carcass data and liver samples were collected. Copper supplementation did not affect ADG ( $P = 0.22$ ). However, the nonsignificant trend for increased ADG and decreased DMI led to a linear increase ( $P = 0.02$ ) feed efficiency (G:F = 0.167, 0.177, and 0.177 for 0, 100, and 200 mg Cu/kg diet DM, respectively). The apparent absorption of Cu decreased quadratically

( $P = 0.07$ ) and the apparent absorption of Mn and Zn were decreased linearly ( $P = 0.03$  and  $P = 0.05$ , respectively) with increased Cu supplementation. Cattle supplemented with 100 or 200 mg Cu/kg diet DM had greater liver Cu concentrations ( $P < 0.01$ ) than cattle that were not supplemented with Cu. There were no treatment effects ( $P > 0.10$ ) on HCW, LM area, USDA yield grade, backfat, or marbling score. Experiment 2 was conducted with 6 ruminally fistulated steers that were fed the same diets as in Exp 1 in a replicated 3 × 3 Latin Square design. Copper supplementation did not affect ( $P > 0.10$ ) ruminal pH or liquid S<sup>2-</sup> concentrations in steers consuming 60% DDGS diets (total dietary S = 0.55%). From 3 to 9 h after feeding, H<sub>2</sub>S gas concentration was decreased in those cattle supplemented with 100 mg Cu/kg diet. Concentration of H<sub>2</sub>S gas did not differ among cattle supplemented with 0 or 200 mg Cu/kg diet DM on 60% DDGS diets. Supplemental Cu improved feed efficiency in cattle consuming diets containing 60% DDGS; however, effects of Cu on rumen S metabolism were minimal even when supplemented at twice the maximum tolerable limit for beef cattle (NRC, 2000).

**Key words:** cattle, copper, dried distillers grains

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## INTRODUCTION

The use of distillers grains can be an excellent feeding strategy for producers to improve gains and decrease feed costs. Ham et al. (1994) reported that dried distillers grains with solubles (DDGS) improved performance when fed at <50% of the diet DM. Feeding cattle diets

that contain >50% DDGS may be problematic for several reasons. The increased concentration of S in DDGS can depress intake, decrease ADG, and have negative health effects (Kandyliis, 1984). In feedlot cattle, diets consisting of 50% DDGS increased the prevalence of polioencephalomalacia (PEM; Buckner et al., 2007). Dietary sulfates are reduced to sulfides in the rumen and sulfides can complex with hydrogen ions to form H<sub>2</sub>S gas. In feedlot cattle, PEM has been identified as S induced when H<sub>2</sub>S is eructated and then inhaled by cattle consuming excess dietary S (Gould et al., 1997; Gould, 1998). In the rumen, Cu and S can precipitate and form Cu sulfides, thereby reducing the availability of both Cu and S to the

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animal (McDowell, 2003). The maximum tolerable level (MTL) of Cu in beef cattle diets is reported to be 100 mg/kg of diet DM (NRC, 2000; McDowell, 2003); however, in diets containing excess S the MTL may be much greater due to reduced availability of Cu. The relationship among Mo–Cu–S complexes, called thiomolybdates, has been well studied in ruminants (McDowell, 2003). However, the efficacy of Cu supplementation in DDGS-based diets to reduce rumen H<sub>2</sub>S and prevent S toxicity has not been reported. We hypothesized that based on its ability to bind S, Cu supplementation would increase growth and feed intake, and decrease rumen H<sub>2</sub>S concentrations in cattle fed DDGS-based diets. The objectives of this study were to determine the effects of Cu supplementation in DDGS-based diets on performance, mineral absorption, ruminal S metabolism, and carcass characteristics of feedlot cattle.

## MATERIALS AND METHODS

All animal procedures were approved by the Agricultural Animal Care and Use Committee of The Ohio State University and followed guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

### Experiment 1

**Animals and Diets.** Eighty-seven yearling, Angus-cross steers and heifers (average initial BW = 238 ± 2.0 kg) were placed on trial March 18, 2009, at the East Agricultural Research Station in Belle Valley, OH. Cattle were blocked by gender and were allotted to 12 pens (8 steers/pen in 3 pens; 7 heifers/pen in 9 pens). Pens (7.3 by 37 m) were constructed of metal gates and cables on concrete and dirt floors in an open-sided barn. Cattle had a minimum of 60 cm of bunk space per animal. Pens within block were randomly allotted to 3 treatments: 1) 0 mg Cu/kg diet DM, 2) 100 mg Cu/kg diet DM, and 3) 200 mg Cu/kg diet DM. Copper was provided from tribasic copper chloride (Micronutrients TBCC, Indianapolis, IN). The remainder of the diet was 60% DDGS, 10% long stem first cutting grass hay, 15% pelleted soy hulls, and 15% supplement (Table 1). The diets were fed for ad libitum intakes for the duration of the 168-d experiment. Cattle were fed once daily at 0900 h. Orts were weighed back daily. Samples of each dietary ingredients were collected every 28 d and were composited for nutrient analysis at the end of the trial.

Steers were implanted with Synovex–S (Fort Dodge Animal Health, Overland Park, KS) and heifers were implanted with Synovex–H (Fort Dodge Animal Health) at the start of the trial (d 0) and were reimplanted with the respective implants on d 112. Cattle were weighed on 2 consecutive d at the beginning and end of the trial

to determine initial and final BW, respectively. Interim BW were taken every 28 d during the trial. Three heifers were removed from the experiment because of PEM-like signs, one from each treatment (2 heifers died and 1 was removed for persistent lack of BW gain).

**Sampling.** On d 112, feed and orts were collected before feeding at 0830 h. Rectal grab samples of feces (~200 g/animal) were collected at 0900 and 1500 h to allow calculation of nutrient digestibility and mineral absorption using Acid Insoluble Ash as an internal digestion marker (2N HCl; Van Keulen and Young, 1977). Apparent DM digestibility was calculated as:

$$\% \text{ DM digestibility} = 100 - [100 \times (\% \text{ AIA in feed DM} / \% \text{ AIA in feces DM})]$$

**Table 1.** Composition of diets in Exp. 1 and 2 for feedlot cattle fed 60% dried distillers grains with solubles (DDGS) with supplemental copper

Item	Cu supplementation, mg Cu/kg diet DM		
	0	100	200
DDGS	60	60	60
Long stem grass hay	10	10	10
Soybean hulls	15	15	15
Supplement			
Ground corn	11.150	11.133	11.116
Limestone	2.790	2.790	2.790
Trace mineral salt <sup>1</sup>	0.457	0.457	0.457
Vitamin A, 30,000 IU/g	0.009	0.009	0.009
Vitamin D, 3,000 IU/g	0.009	0.009	0.009
Vitamin E, 44 IU/g	0.027	0.027	0.027
Selenium, 201 mg/g	0.046	0.046	0.046
Rumensin 80 <sup>2</sup>	0.016	0.016	0.016
Tylan 10 <sup>3</sup>	0.046	0.046	0.046
Animal–Vegetable fat blend	0.450	0.450	0.450
Copper chloride <sup>4</sup>	0.000	0.017	0.034
Analyzed composition			
NDF, %	36.12	36.08	36.03
ADF, %	20.39	20.35	20.28
CP, %	19.2	19.2	19.1
Ether extract, %	7.9	7.9	7.9
Ca, %	1.10	1.38	1.10
P, %	0.48	0.48	0.48
Mg, %	0.26	0.26	0.26
S <sup>5</sup> , %	0.55	0.55	0.55
Mo, mg/kg	1.38	1.42	1.43
Mn, mg/kg	29.98	30.73	30.44
Zn, mg/kg	77.80	79.51	81.78
Cu, mg/kg	6.40	112.38	188.34

<sup>1</sup>Included: 95% NaCl; 0.35% Zn, as ZnO; 0.28% Mn, as MnO<sub>2</sub>; 0.175% Fe, as FeCO<sub>3</sub>; 0.040% Cu, as Cu<sub>2</sub>O; 0.007% I, as Ca<sub>5</sub>(IO<sub>6</sub>)<sub>2</sub>; 0.007% Co, as CoCO<sub>3</sub>.

<sup>2</sup>Rumensin, 176 g/kg, Elanco (Greenfield, IN).

<sup>3</sup>Tylan, 22 g/kg, Elanco.

<sup>4</sup>Formulated to include 0, 100, and 200 mg Cu/kg diet DM; Micronutrients TBCC (Indianapolis, IN).

<sup>5</sup>S of the DDGS was 0.72%.

Apparent nutrient digestibility (or mineral absorption) was calculated as:

$$\% \text{ nutrient digestibility} = 100 - [100 \times (\% \text{ AIA in feed DM} / \% \text{ AIA in feces DM}) \times (\% \text{ nutrient in feces DM} / \% \text{ nutrient in feed DM})]$$

Feces from all cattle within a pen were composited across both sampling times into 1 sample/pen and then stored at  $-20^{\circ}\text{C}$  until later analysis.

Three cattle closest to the mean BW from each pen were sent to the abattoir for harvest at the end of the feeding trial (d 168) so that carcass data and liver samples could be collected on this subset. Cattle were harvested at the Ohio Department of Rehabilitation and Corrections slaughter facility in Orient, OH. After evisceration, a sample was taken from the same location on each liver, put on ice, and taken back to the lab for later analysis.

Feed, orts, fecal, and liver samples were all freeze dried (Freeze Dryer 8, Labconco, Kansas City, MO). Feed, orts, and feces were then ground using a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Liver samples were ground in a food processor that was cleaned with water and then ethanol between each sample to prevent mineral contamination between samples. All samples were analyzed for DM (24 h at  $100^{\circ}\text{C}$ ). Freeze-dried samples underwent perchloric acid digestion and inductively coupled plasma atomic emission spectroscopy analysis of complete minerals (AOAC, 1988). Feed samples were analyzed for ADF and NDF (using Ankom Technology Method 5 and 6, respectively; Ankom<sup>200</sup> Fiber Analyzer, Ankom Technology, Fairport, NY), CP (macro Kjeldahl  $\text{N} \times 6.25$ ), and crude fat (using the ether extract method; Ankom Technology, Fairport, NY).

**Statistical Analysis.** The experimental design for this study was a randomized complete block design. Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model used was:

$$Y_{ijk} = \mu + b_i + M_k + e_{ijk}$$

where  $Y_{ijk}$  = response variable;  $\mu$  = mean;  $b_i$  = the fixed effect of block (df = 1);  $M_k$  = the fixed effect of Cu inclusion (df = 2);  $e_{ijk}$  = the experimental error (df = 8). Pen was the experimental unit. Linear and quadratic contrasts were analyzed to examine the effect of increasing Cu supplementation on dependent variables. Significance was declared at  $P < 0.10$ .

## Experiment 2

**Animals and Diets.** Six Angus-cross ruminally fistulated steers (initial BW =  $532 \pm 35$  kg) were placed on trial at the Ohio Agriculture Research and Development

Center feedlot in Wooster, OH. Steers were fed the same dietary treatments used in Exp. 1. The diets were offered ad libitum in a replicated  $3 \times 3$  Latin Square Design. Dietary treatment sequences were assigned according to procedures described by Patterson and Lucas (1962).

Cattle were housed in individual pens. Pens were 2.6 by 1.5 m and consisted of metal gates and a slatted concrete floor. Cattle were fed hand mixed diets daily at 0800 h. Dietary ingredient samples were collected at the beginning of each period to determine DM adjustments. Samples of baled hay were taken with a corer. Each period consisted of a 14-d diet adjustment phase followed by 1 d of rumen collections (0, 3, 6, and 9 h postfeeding) for ruminal hydrogen sulfide gas ( $\text{H}_2\text{S}$ ), liquid sulfide ( $\text{S}^{2-}$ ), and pH determination.

**Sampling.** Feed and orts samples were collected on d 15 of each period at 0730 h, directly before rumen sampling. A sample of orts and 0.45 kg of each diet component were saved for later analysis of minerals as described in Exp. 1.

Rumen gas was sampled similar to the technique that was validated by Gould et al. (1997), except that gas was sampled through the cannula cap via puncture with a 10-gauge needle. The  $\text{H}_2\text{S}$  concentration was measured via hydrogen sulfide precision gas detector tubes (No. 120SF, Sensidyne, Mülheim, Germany) attached to a calibrated gas detection pump (Model AP-20S, Sensidyne). The concentration of  $\text{H}_2\text{S}$  was recorded from the detector tube by the same individual for each sampling.

Measurements of pH and liquid  $\text{S}^{2-}$  were taken within 2 min after fluid collection. Rumen fluid samples were strained through 2 layers of cheesecloth. A dual meter was used to measure pH and  $\text{S}^{2-}$  (Accumet excel XL25 dual channel pH/ion meter; Fisher Scientific, Waltham, MA). To measure pH, the electrode (Accumet pH/ATC polypropylene body liquid-filled combination electrode with Ag/AgCl reference with BNC mini connector; Fisher Scientific) was submersed in the strained rumen fluid. To measure liquid  $\text{S}^{2-}$ , 25 mL of strained rumen liquid was mixed with 25 mL of sulfide antioxidant buffer (SAOB; Fisher Scientific Cat. No. 13-641-882) to stabilize the  $\text{S}^{2-}$  ions. The samples were shaken vigorously and then  $\text{S}^{2-}$  was measured via sulfide electrode (Accumet silver/sulfide combination electrode; Fisher Scientific).

**Statistical Analysis.** The experimental design was a replicated  $3 \times 3$  Latin Square. Repeated measures were used to analyze the effects of sampling time on ruminal  $\text{H}_2\text{S}$ , liquid  $\text{S}^{2-}$ , and pH, using the covariate structure for compound symmetry. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc.). The model was:

$$Y_{ijklm} = \mu + S_i + c_{j(i)} + p_k + M_l + T_m + (\text{TM})_{ml} + c_{i(m)} + e_{ijklm}$$

**Table 2.** Effect of Cu supplementation on feedlot performance of cattle fed diets containing 60% dried distillers grains with solubles in Exp. 1<sup>1</sup>

Item	Cu supplementation, mg Cu/kg diet DM			SE	P-value	
	0	100	200		Linear	Quadratic
No. animal (pen)	28 (4)	28 (4)	28 (4)	–	–	–
DOF <sup>2</sup>	168	168	168	–	–	–
Initial BW, kg	240	236	239	2.4	0.47	0.04
Final BW, kg	492	497	500	9.5	0.24	0.84
ADG, kg	1.50	1.55	1.55	0.061	0.22	0.46
DMI, kg	9.06	8.89	8.95	0.370	0.65	0.56
G:F	0.167	0.177	0.177	0.003	0.02	0.14

<sup>1</sup>Data are least squares means.<sup>2</sup>DOF = days on feed.

where,  $Y_{ijklm}$  = response variable;  $\mu$  = mean;  $S_i$  = the fixed effect of square ( $df = 1$ );  $c_{j(i)}$  = the random effect of calf nested within square ( $df = 4$ );  $p_k$  = the random effect of period ( $df = 2$ );  $M_1$  = the fixed effect of copper inclusion ( $df = 2$ );  $T_m$  = the fixed effect repeated of time of collection ( $df = 3$ );  $(TM)_{ml}$  = the fixed effect of time  $\times$  copper interaction ( $df = 6$ );  $c_{i(m)}$  = the random effect of calf nested within time ( $df = 12$ ); and  $e_{ijklm}$  = the experimental error ( $df = 42$ ). Significance was declared at  $P < 0.10$ .

## RESULTS AND DISCUSSION

### Experiment 1

There were no effects ( $P > 0.10$ ) of Cu supplementation on final BW, ADG, or DM intake (Table 2). However, the nonsignificant trend for increased ADG and decreased DMI led to a linear increase ( $P = 0.02$ ) in G:F in cattle that were supplemented with increasing dietary concentrations of Cu. Improved G:F has been reported when diets containing elevated Cu are fed to swine due to antibiotic-like effects in the intestine (Apgar et al., 1995; Cromwell et al., 1998), although the mechanism of action is not fully understood. The 6% improvement in feed efficiency noted in this group of cattle could be due to Cu alleviating some of the toxic effects of elevated dietary S. However, the typical Cu sulfide formed in the rumen is a monosulfide (Qi et al., 1993). Therefore, in the present experiment, supplementation with 100 mg Cu/kg diet DM could bind up to a maximum of 2.04% of the dietary S, reducing the potentially available S for absorption only slightly (0.55% to 0.54% of diet DM). Similarly, supplementation with 200 mg Cu/kg diet DM (analyzed at 188.3 mg Cu/kg diet DM) could hypothetically bind a maximum of 3.5% of S, making the potentially available dietary S equal to 0.525%. Although this reduction seems small, a meta analysis by Vanness et al. (2009) showed cattle consuming diets containing 0.47 to 0.56% S had a 0.35% incidence of PEM; however, once dietary S was above 0.56%, the incidence

**Table 3.** Effect of Cu supplementation on apparent digestion of DM and apparent absorption of nutrients in feedlot cattle fed diets containing 60% dried distillers grains with solubles in Exp. 1<sup>1</sup>

Item	Cu supplementation, mg Cu/kg diet DM			SE	P-value	
	0	100	200		Linear	Quadratic
DM, %	75.68	77.89	69.53	2.87	0.16	0.17
P, %	73.83	75.30	65.52	3.20	0.10	0.18
Ca, %	45.93	47.94	30.60	5.01	0.06	0.15
Mg, %	42.10	49.50	30.86	6.68	0.26	0.15
S, %	84.51	84.94	79.43	2.03	0.11	0.26
Cu, %	21.08	42.12	10.75	10.58	0.51	0.07
Mn, %	37.25	29.68	7.55	8.27	0.03	0.49
Mo, %	30.71	35.86	11.47	8.22	0.13	0.18
Zn, %	43.38	51.13	21.64	6.92	0.05	0.06

<sup>1</sup>Data are least squares means.

of PEM rose dramatically to 6.06%. Therefore, very small reductions in available dietary S could reduce the risk for PEM. The Mo concentration in the diet was ~1.5 mg Mo/kg DM; therefore, Mo was not likely a significant factor in the binding of Cu and S.

Increasing Cu in the diet linearly decreased ( $P < 0.10$ ) the apparent absorption of P, Ca, Mn, and Zn, and quadratically decreased ( $P = 0.07$ ) the apparent absorption of Cu (Table 3). Use of AIA in a feedlot setting is not an ideal marker for measures of mineral metabolism. Intake of minerals from nonfeed sources could not be controlled and no measure of urinary excretion was possible. Despite these limitations, Standish et al. (1971) reported very similar values for Ca, Cu, Zn, Mn, and Mg absorption in growing beef steers. Although Standish et al. (1971) reported much less P absorption (27 to 42%), they fed diets with excess Fe and suggested that P absorption was reduced due to binding with the excess Fe. Similar absorption values, as reported in Table 3 for Ca, P and Mg, were also reported by Schingoethe et al. (1980) in Holstein steers. Although their absorption values were slightly less than those reported in the present study, their diets contained 37% less Ca, 8% less P, and 43% less Mg. As stated previously, increased concentration of Cu in the experimental diets used in the present experiment decreased ( $P \leq 0.05$ ) apparent absorption of Zn and Mn. In the duodenum, Cu, Zn, and Mn are carried across the small intestinal lumen by the same transporter, divalent metal transporter 1 (**DMT1**; Gunshin et al., 1997). In our study, Cu that did not bind with S may have competed with Zn and Mn for uptake, thereby reducing the apparent absorption of Zn and Mn. However, DMT1 is not the only transporter of Cu. Copper Transporter 1 (**CTR1**) is also responsible for Cu uptake in the small intestine and it has been suggested that increased concentrations of dietary Cu may cause CTR1 to relocate from the plasma

**Table 4.** Effect of Cu supplementation on liver mineral concentrations (DM basis) from feedlot cattle fed diets containing 60% dried distillers grains with solubles in Exp. 1<sup>1</sup>

Item	Cu supplementation, mg Cu/kg diet DM			SE	P-value	
	0	100	200		Linear	Quadratic
No. animal (pen)	12 (4)	12 (4)	12 (4)	–	–	–
Minerals, µg/g						
Cu	86.29	708.2	933.3	74.87	<0.01	0.06
Fe	222.7	221.5	211.0	8.88	0.38	0.68
Zn	129.8	126.5	126.9	2.13	0.37	0.49
S	6,255.0	6,845.2	6,817.7	98.0	<0.01	0.03
Mn	10.99	11.87	12.24	0.34	0.03	0.56
Mo	3.49	3.17	3.21	0.15	0.23	0.34
P	10,341	10,869	10,723	161.2	0.13	0.12
K	8,795	9,223	9,592	211.9	0.03	0.91
Ca	116.8	126.5	139.1	3.64	<0.01	0.75
Mg	583.8	608.7	628.8	11.4	0.02	0.86

<sup>1</sup>Data are least squares means.

membrane to the cytosol to decrease Cu accumulation (Kim et al., 2008). This relocation may explain the quadratic decrease ( $P = 0.07$ ) in Cu absorption observed in our study. Increasing dietary Cu did not affect ( $P > 0.10$ ) apparent absorption of S. Consequently, Cu supplementation at twice the NRC (2000) MTL for beef cattle was not sufficient to affect our measurement of S absorption.

Increasing Cu supplementation linearly increased ( $P \leq 0.03$ ) accumulation of Ca, Cu, S, Mg, and Mn in the liver (Table 4), and this response occurred despite no increase in apparent absorption of these minerals with increasing supplemental Cu. Increased Cu concentrations in the liver with increasing dietary Cu has been researched previously (Cousins, 1985; Luza and Speisky, 1996; Engle and Spears, 2000). The increase in liver Cu concentrations with Cu supplementation suggests these cattle may have been approaching risk for Cu toxicosis (NRC 2005). Increasing Cu supplementation increased S accumulation in the liver. This effect of Cu on liver S has not previously been reported. In the rumen, Cu and S precipitate to form CuS, which is assumed to be unavailable (McDowell, 2003). Increased dietary Cu and S can also cause CuS to precipitate in tissues after absorption (Qi et al., 1993). Therefore, the precipitation of CuS may have caused the increase ( $P < 0.01$ ) in S in the liver.

There was no effect ( $P > 0.10$ ) of Cu supplementation on HCW, LM area, backfat, USDA yield grade, or marbling score (Table 5). A previous study demonstrated that 20 mg Cu/kg diet tended to decrease backfat (Engle et al., 2000); however, we were not able to demonstrate this effect of Cu at 5 to 10 times supplementation rate of the previous study.

**Table 5.** Effects of Cu supplementation on carcass characteristics of feedlot cattle fed diets containing 60% dried distillers grains with solubles in Exp. 1<sup>1</sup>

Item	Cu supplementation, mg Cu/kg diet DM			SE	P-value	
	0	100	200		Linear	Quadratic
No. animal (pen)	12 (4)	12 (4)	12 (4)	–	–	–
HCW, kg	284	290	293	4.58	0.17	0.73
BF <sup>2</sup> , cm	1.30	1.28	1.33	0.12	0.85	0.78
LM area, cm <sup>2</sup>	73.45	76.06	74.84	1.37	0.47	0.26
YG <sup>2</sup>	2.92	2.81	2.95	0.13	0.85	0.45
Marbling score <sup>3</sup>	591	589	576	26.39	0.68	0.88

<sup>1</sup>Data are least squares means.

<sup>2</sup>BF = backfat; YG = USDA yield grade.

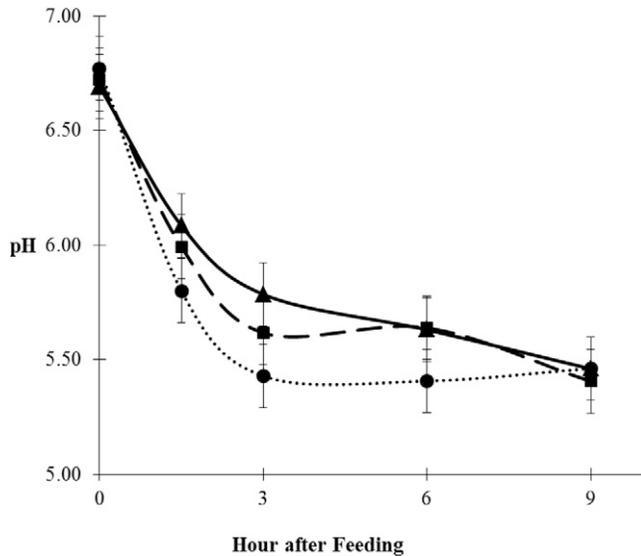
<sup>3</sup>The scale used for marbling score was 400 = slight, 500 = small, 600 = moderate.

## Experiment 2

Increasing dietary Cu supplementation resulted in a linear ( $P < 0.01$ ) increase in Cu intake as would be expected (0.14, 1.36, 2.17 g Cu/d for 0, 100, and 200 mg Cu/kg diet, respectively). As observed in Exp. 1, Cu supplementation did not affect ( $P > 0.18$ ) DMI (11.1, 11.5, and 11.0 for 0, 100, and 200 mg Cu/kg diet, respectively). Intakes of other minerals were not affected ( $P > 0.17$ ) by Cu supplementation (data not shown).

There was no effect ( $P > 0.10$ ) of supplemental Cu on rumen pH (Figure 1) or liquid S<sup>2-</sup> concentration (Figure 2). Regardless of dietary Cu concentration, steers had a rumen pH of >6.5 directly before feeding and within 3 h after feeding, rumen pH decreased approximately one full pH unit (Figure 1). Rumen pH of steers fed a 92.5% concentrate diet once daily decreased during the 12 h after feeding from ~6.2 to 5.0 (Cooper et al., 1998). Until recently, it has been postulated that DDGS would not affect rumen pH as dramatically as corn because of the increased fiber content and lack of readily fermentable starch contained in DDGS (Schingoethe, 2004; Leupp et al., 2009). However, Vander Pol et al. (2009) reported that cattle consuming 40% wet distillers grains with solubles had decreased rumen pH compared with cattle fed corn diets. Our data indicate that the rumen pH in cattle fed 60% DDGS diets is more typical of cattle fed grain-based diets than high fiber diets. Felix and Loerch (2011) reported that 10% haylage supplementation was an effective strategy to increase rumen pH in cattle fed 60% DDGS diets.

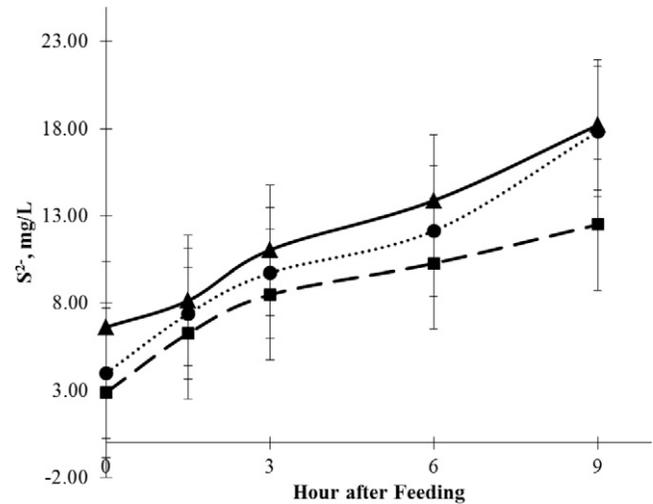
In the rumen, Cu and S interact to form insoluble Cu sulfides (McDowell, 2003). In our experiment, S<sup>2-</sup> was measured to determine whether Cu would bind S from the 60% DDGS diets and keep more S in the liquid phase. We had hypothesized Cu would bind S, thereby decreasing H<sub>2</sub>S gas concentrations and increasing S<sup>2-</sup> in the rumen liquid. Reducing H<sub>2</sub>S gas concentrations would reduce risk of PEM and absorption of S would allow detoxification by the



**Figure 1.** Effects of Cu supplementation on rumen pH over time in cattle fed diets containing 60% dried distillers grains with solubles. Copper was supplemented to steers at either 0 mg Cu/kg diet DM (●), 100 mg Cu/kg diet DM (■), or 200 mg Cu/kg diet DM (▲). Effects of Cu supplementation were not detected ( $P > 0.10$ ). Error bars = SE.

blood (Evans, 1967) and liver (Anderson, 1956). However, there was no effect ( $P > 0.10$ ) of Cu supplementation on liquid  $S^{2-}$  concentrations (Figure 2). The  $S^{2-}$  concentration in the liquid was least before feeding and increased through 9 h postfeeding, reflecting the consumption of S. Some of the S consumed may have originated from sulfuric acid ( $H_2SO_4$ ) present in the DDGS. Sulfuric acid is typically used in ethanol production to control pH during enzymatic hydrolysis of corn starch and prevent bacterial growth in the fermentation process (McAloon et al., 2000). Felix and Loerch (2011) reported that the titratable acidity of DDGS was 505 mL of 1 M NaOH/kg of DDGS. Approximately one-half of the S in the DDGS used in the present experiment was calculated to originate from  $H_2SO_4$  and would be readily available to sulfate-reducing bacteria.

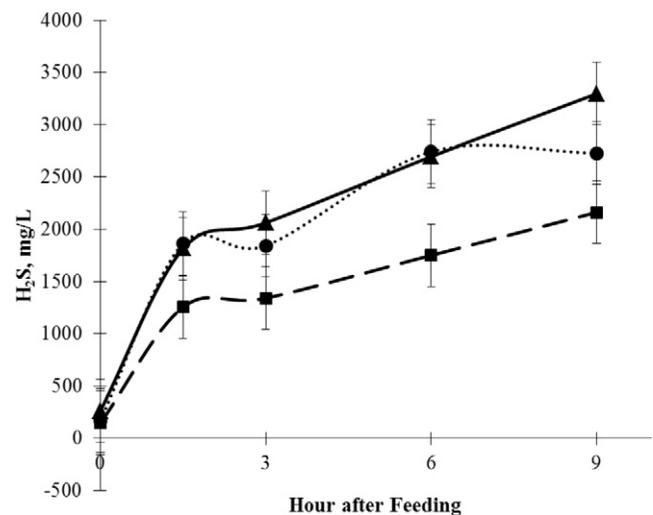
The concentration of  $H_2S$  was also smallest (300 to 750 mg/L) before feeding but rose rapidly to 2,250 mg/L by 3 h after feeding and remained increased for up to 9 h after feeding (Figure 3). At 3 h after feeding, 100 mg Cu/kg diet DM decreased ( $P = 0.07$ )  $H_2S$  concentration when compared with 0 or 200 mg Cu/kg diet DM. We have no explanation for the lack of linearity in this response. The  $H_2S$  concentrations measured were highly dependent on time after feeding. This suggests a single measurement during the day may not accurately reflect  $H_2S$  concentration in ruminal gas and the risk of PEM. Gould (1998) stated that  $>2,000$  mg/L  $H_2S$  typically preceded observed cases of PEM. Although PEM was not observed in the fistulated cattle on this experiment, 3 heifers were removed from the feedlot study; 1 due to morbidity and 2 due to clinical symptoms of PEM.



**Figure 2.** Effects of Cu supplementation on rumen liquid sulfide ( $S^{2-}$ ) over time in cattle fed diets containing 60% dried distillers grains with solubles. Copper was supplemented to steers at either 0 mg Cu/kg diet DM (●), 100 mg Cu/kg diet DM (■), or 200 mg Cu/kg diet DM (▲). Effects of Cu supplementation were not detected ( $P > 0.10$ ). Error bars = SE.

### Conclusions

In 60% DDGS-based diets, supplemental Cu as tribasic Cu chloride improved feed efficiency without altering other measures of performance or carcass characteristics. Copper supplementation decreased the apparent absorption of Cu, Zn, and Mn; however, it increased liver Cu and S accumulation. Supplementing 200 mg Cu/kg diet DM to cattle fed 60% DDGS diets did not affect rumen S metabolism as measured by  $H_2S$  in the gas and  $S^{2-}$  in the liquid of the rumen. Although fed at 2 times the MTL, Cu supplementation does not appear to be an effective means of reducing ruminal  $H_2S$  production and the toxic effects of S on DM intake or PEM.



**Figure 3.** Effects of Cu supplementation on rumen hydrogen sulfide gas ( $H_2S$ ) concentration over time in cattle fed diets containing 60% dried distillers grains with solubles. Copper was supplemented to steers at either 0 mg Cu/kg diet DM (●), 100 mg Cu/kg diet DM (■), or 200 mg Cu/kg diet DM (▲). Time  $\times$  Cu supplementation interaction ( $P < 0.05$ ). Error bars = SE.

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