

# The Effects of Copper Source and Concentration on Lipid Metabolism, Carcass Characteristics, and Fatty Acid Profile in Growing and Finishing Angus Steers

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## SUMMARY

Forty-eight individually fed Angus steers (body weight 484 lb ± 20) were utilized to investigate the effects of copper (Cu) source and concentration on lipid metabolism and carcass quality. Dietary treatments consisted of 1) control (no supplemental Cu); 2) 10 ppm Cu from CuSO<sub>4</sub>; 3) 10 ppm Cu from Availa Cu; 4) 20 ppm Cu from CuSO<sub>4</sub>; and 5) 20 ppm Cu from Availa Cu. Ceruloplasmin activity was higher ( $P < 0.04$ ) for steers receiving 20 ppm Cu from Availa relative to steers receiving 20 ppm Cu from CuSO<sub>4</sub>. Plasma non-esterified fatty acids were similar across treatments. Steers receiving 10 ppm Cu from Availa Cu had higher ( $P < 0.02$ ) plasma cholesterol concentrations relative to steers receiving 10 ppm Cu from CuSO<sub>4</sub>. Copper supplemented steers tended ( $P < 0.09$ ) to have lower plasma triglyceride (TG) concentrations than control steers, and steers receiving 20 ppm Cu from Availa Cu had lower TG concentrations than steers supplemented with 20 ppm Cu from CuSO<sub>4</sub>. Fatty acid profile of longissimus muscle was similar across treatments. Steers fed 10 ppm Cu from Availa Cu tended to have heavier hot carcass weights ( $P < 0.10$ ), larger ribeye areas ( $P < 0.11$ ) and greater backfat deposition ( $P < 0.11$ ) than steers supplemented with iso-amounts from CuSO<sub>4</sub>. Furthermore, Cu supplementation tended ( $P < 0.06$ ) to increase basal and increased ( $P < 0.04$ ) epinephrine stimulated lipolytic rates in subcutaneous adipose tissue. The results of this study suggest that

Cu supplementation has minimal effects on blood and tissue lipid profile. However, it appears that Cu may play a role in lipid metabolism in subcutaneous adipose tissue.

**Key words:** Steer, Copper, Lipid Metabolism, Fatty Acid

## INTRODUCTION

Several studies have indicated that copper plays a beneficial role in altering lipid composition in animals. Research involving chickens has shown that supplemental dietary copper (Cu) reduces plasma and breast muscle cholesterol concentrations (Konjufca et al., 1997). Also, rat and swine studies have indicated that Cu reduces backfat as well as lowers blood lipids.

The effects of copper on lipid metabolism in cattle are more complex. Ward and Spears (1997) reported that Cu supplementation to finishing steers decrease backfat depth. Recently, Engle et al. (2000b) reported that steers supplemented with Cu had lower serum cholesterol concentrations and less backfat relative to control steers, and that marbling was unaffected by the reduction in backfat.

Furthermore, Cu has been associated with increasing the conjugated linoleic acid (CLA) concentration in longissimus muscle as well as the unsaturated fatty acid: saturated fatty acid ratio (Engle et al. 2000a). Conjugated linoleic acid has been shown to have anticarcinogenic, antiobesity, antidiabetic, and antiatherogenic effects in laboratory rats (Pariza et al., 1999). The benefits from CLA along with the general benefits of a lower saturated fat product are of particular interest to meat animal producers. Therefore, the objective of this study was to confirm the effects of Cu on blood and tissue lipids and backfat, as well as to investigate the effects of Cu on subcutaneous adipose lipid metabolism.

## MATERIALS AND METHODS

### Cattle Management.

Research, handling, and sampling methods used in this study were approved by the Colorado State University Animal Care and Use Committee.

Forty-eight purebred Angus steers (body weight 484 lb ± 20) from the Colorado State University (CSU) Beef Improvement Center were transported approximately 125 mi to our feedlot facility. Upon arrival, steers were weighed on two consecutive days, vaccinated with Bovishield 4+/L5 and Cattlemaster 4, treated for parasites with Dectomax (Pfizer Animal Health, Exton, PA), bled via jugular veinipuncture, implanted with Ralgro® (Scheng Canada Inc., Intervet, Millsboro, DE), and liver biopsies were obtained from each animal and analyzed for Cu. Steers were then stratified by body weight and liver copper concentration and randomly assigned to one of five groups. Groups were then randomly assigned to treatments. Treatments consisted of: 1) control (no supplemental Cu); 2) 10 ppm Cu from CuSO<sub>4</sub>; 3) 10 ppm Cu from Availa Cu; 4) 20 ppm Cu from CuSO<sub>4</sub>; and 5) 20 ppm Cu from Availa Cu. Steers were fed a corn-alfalfa-based growing diet for 56 d (45% chopped alfalfa hay, 47% corn grain, 7% protein supplement, and 1% mineral mix<sup>1</sup>). Steers were housed in individual pens with automatic waterers. Throughout the growing phase steers and were weighed and bled and feed refusals were calculated every 28 days.

After the 56-day growing phase, steers were then switched to a high concentrate diet for 145 d (6% chopped alfalfa hay, 86% corn grain, 7% protein supplement, and the 1% mineral mix) and re-implanted with Revalor S (Intervet, Millsboro, DE).

Steers remained on the finishing ration for 145 days. Throughout this period, blood samples, feed refusals, and body weights were taken every 28

<sup>1</sup> Provided per pound of diet: 66 mg Zn as ZnSO<sub>4</sub>, 44 mg of Mn as MnSO<sub>4</sub>, 1.1 mg of I as EDDI, and 0.22 mg of Co as CoCO<sub>3</sub>

days. On day 74 of the finishing phase adipose tissue biopsies were taken from 15 steers (three steers/treatment group). A portion of adipose tissue was surgically removed from the right side of the tail head, and samples were used to measure in vitro lipolytic rates.

On d 145 of the finishing phase, steers were transported (approximately 31 mi) to a commercial abattoir. After slaughter, hot carcass weights were obtained and carcasses were graded 48 hours post harvesting. After grading, a thin slice of the longissimus muscle was obtained from each carcass for fatty acid analysis. Samples were placed on ice, transported to the laboratory, and frozen at  $-112^{\circ}\text{F}$  until analyzed for fatty acid profile.

#### **Analytical Procedures.**

Ceruloplasmin activity (an indicator of Cu status) in plasma was analyzed according to the procedure described by O.B. Houchin (1958). The remaining plasma was analyzed for total cholesterol, non-esterified fatty acids (NEFAs), and triglycerides (TG) via enzymatic and colorimetric methods (Sigma Chemical Co, 2000, Wako Chemical Co., 1995, and Sigma Chemical Co., 1998; respectively).

Subcutaneous adipose biopsy tissue samples were immediately dissected into six 200 mg segments. Three segments were placed into a basal buffering solution as described by Pothoven et al. (1975). The remaining three segments were placed in an epinephrine stimulated buffering solution. Samples were incubated, tissue was reweighed post-incubation, and solution was decanted for further analysis. Solution and tissue samples were analyzed for glycerol concentrations via the method described by Laurell and Tibbling (1966).

Longissimus muscle was dissected of any visible external fat and the remaining tissue was finely diced and mixed thoroughly. A representative subsample of approximately 1 g was taken for lipid extraction according to the Bligh and Dyer procedure using a 2:1 chloroform: methanol solution

(Bligh and Dyer, 1959). The lipid fraction was dried under nitrogen and then placed in a  $212^{\circ}\text{F}$  oven for 4 hours. Vials were cooled in a desiccator, and their weights were compared to the dry weights of the empty vials to determine the percent lipid extracted from the longissimus muscle.

Fatty acid composition of longissimus muscle was determined by gas chromatography using a Hewlett Packard Model 6890A Series II gas chromatograph fixed with a series 7683 injector and flame ionization detector. The instrument was equipped with a 100-m x 0.25-mm (i.d.) fused silica capillary column (SP<sup>TM</sup>-2560 Supelco Inc. Bellefonte, PA). Methyl ester derivatives of the fatty acids were prepared using a combination of NaOCH<sub>3</sub> followed by HCl/methanol as described by Kramer et al. (1997). Fatty acid methyl ester preparations were injected (1 $\mu\text{L}$ ) using the split mode. The carrier gas was helium, and the split ratio was 100:1 at  $356^{\circ}\text{F}$ . The oven temperature was programmed from an initial temperature of  $284^{\circ}\text{F}$  (0 min) to a final temperature of  $437^{\circ}\text{F}$  at the rate of  $37^{\circ}\text{F}/\text{min}$ . The final temperature was held for 18 min. Chromatograms were recorded with a computing integrator (Agilent Technologies). Standard fatty acid methyl ester mixtures were used to calibrate the gas chromatograph system using reference standards KEL-FIM-FAME-5 (Matreya Inc., PA). Identification of the fatty acids was made by comparing the relative retention times of fatty acid methyl ester peaks from samples with those of standards. These were calculated as normalized area percentages of fatty acids.

#### **Statistical Analysis.**

Statistical analyses of data were analyzed using the Mixed Procedure of SAS (1989). When a significant ( $P < 0.05$ ) treatment effect was noted, differences between treatment means were determined using single degree of freedom contrasts. Comparisons were made for 1) control vs. Cu supplemented, 2) 10 ppm Cu vs. 20

ppm Cu, 3) 10 ppm Cu from CuSO<sub>4</sub> vs. 10 ppm Cu from Availa<sup>®</sup> Cu and 4) 20 ppm Cu from CuSO<sub>4</sub> vs. 20 ppm Cu from Availa<sup>®</sup> Cu. Trends in data are noted for  $P \leq 0.10$  as there may have been a greater response seen with a larger treatment groups and number of animals.

## **RESULTS AND DISCUSSION**

During the study one steer from the 10 ppm Cu from CuSO<sub>4</sub> treatment died from bloat and all data pertaining to this animal was removed from statistical analysis.

Steer performance data and liver biopsy mineral values will be reported elsewhere (Dorton et al., 2002). There was an overall treatment effect ( $P < 0.05$ ) for ceruloplasmin (Cp) activity. Control steers tended ( $P < 0.12$ ) to have lower Cp activity than Cu supplemented steers. Steers receiving 20 ppm Cu from Availa Cu had higher ( $P < 0.03$ ) ceruloplasmin activities than steers receiving 20 ppm Cu from CuSO<sub>4</sub> (data not shown).

Blood lipid composition was comprised of total cholesterol, NEFAs and TG (Table 1). There were no differences between treatment groups as well as no apparent treatment effect for NEFAs. However, Cu supplemented steers tended ( $P < 0.11$ ) to have higher total cholesterol concentrations than control steers. Steers receiving 10 ppm Cu from Availa Cu had higher ( $P < 0.02$ ) total plasma cholesterol concentrations than steers receiving 10 ppm Cu from CuSO<sub>4</sub>.

Plasma TG concentrations tended to be lower ( $P < 0.09$ ) in the Cu supplemented steers relative the controls (Table 1). Steers receiving 10 ppm Cu from Availa Cu tended ( $P < 0.09$ ) to have lower plasma TG concentrations relative to steers supplemented with 10 ppm Cu from CuSO<sub>4</sub>. Furthermore, steers receiving 20 ppm Cu from Availa Cu had lower ( $P < 0.03$ ) plasma TG concentrations relative to steers supplemented with 10 ppm Cu from CuSO<sub>4</sub>.

Carcass data is shown in Table 2. Steers receiving 10 ppm Cu from Availa Cu tended to have greater 12<sup>th</sup>

rib backfat depth ( $P < 0.10$ ), a larger ribeye area ( $P < 0.11$ ), and heavier hot carcass weights ( $P < 0.10$ ) than steers supplemented with 10 ppm Cu from  $\text{CuSO}_4$ . Dressing percentage was higher ( $P < 0.02$ ) in steers receiving 20 ppm Cu from Availa Cu relative to steers receiving iso-amounts of Cu from  $\text{CuSO}_4$ .

Fatty acid profile of longissimus muscle was unaffected by treatment (data not shown;  $P > 0.28$ ). This is in contrast to previously reported research. Interestingly, Cu tended ( $P < 0.06$ ) to increase basal and increased ( $P < 0.04$ ) epinephrine stimulated lipolytic rates in subcutaneous adipose tissue (Table 3). This is in agreement with earlier research reported in sheep (Sinnott-Smith and Woolliams, 1987).

### IMPLICATIONS

A majority of the current research has shown that copper supplementation is beneficial to cattle, not simply for maintaining proper health but also in aiding immune function and lipid metabolism. The results of this study indicate that Cu supplementation had minimal effect on blood lipid concentrations and longissimus muscle fatty acid profile. However, it appears that Cu may play a role in lipid metabolism in subcutaneous adipose tissue. Further research is needed to determine the metabolic role of Cu in subcutaneous adipose tissue metabolism.

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Table 1: Effects of copper source and concentration on blood lipid profile

Variable	Dietary Treatments					SEM	Contrasts ( $P < $ )			
	Control	10 ppm CuSO4	10 ppm Availa Cu	20 ppm CuSO4	20 ppm Availa Cu		Control vs. Copper	10 ppm vs. 20 ppm	CuSO4 vs. Availa Cu at 10 ppm	CuSO4 vs. Availa Cu at 20 ppm
Total	75.30	75.78	88.53	80.57	79.40	3.882	0.18	0.58	0.02	0.84
Cholesterol (mg/dL)										
Non-esterified Fatty Acids (mmol/L)	0.22	0.22	0.21	0.22	0.19	0.018	0.53	0.80	0.65	0.25
Triglycerides (mg/dL)	22.62	22.5	21.45	22.33	20.97	0.437	0.09	0.47	0.09	0.03



Table 2. The effects of copper source and concentration on carcass performance

Variable	Dietary Treatments					SEM	Contrasts (P<)			
	Control	10 ppm CuSO <sub>4</sub>	10 ppm Availa Cu	20 ppm CuSO <sub>4</sub>	20 ppm Availa Cu		Control vs. Copper	10 ppm vs. 20 ppm	CuSO <sub>4</sub> vs. Availa Cu at 10 ppm	CuSO <sub>4</sub> vs. Availa Cu at 20 ppm
12th rib backfat (in)	0.57	0.50	0.59	0.46	0.52	0.041	0.32	0.21	0.10	0.30
Marbling <sup>a</sup>	412.78	406.33	442.22	379.44	386.67	24.109	0.74	0.10	0.30	0.83
Ribeye area (in <sup>2</sup> )	11.52	10.97	11.62	11.00	11.33	0.283	0.36	0.66	0.11	0.41
Hot carcass weight (lbs)	707.50	689.46	729.89	702.44	714.45	13.000	0.79	0.66	0.10	0.52
Dressing Percent	58.44	58.20	58.92	57.72	59.13	0.004	0.92	0.75	0.23	0.02
KPH <sup>b</sup> fat (%)	1.81	1.83	1.92	1.97	2.00	0.077	0.16	0.16	0.45	0.80

<sup>a</sup> 300=slight; 400=small; 500=modest

Table 3. Effects of copper concentration and source on basal and stimulated rates of lipolysis in subcutaneous adipose tissue in finishing steers

Variable	Dietary Treatments					SEM	Contrasts (P<)				
	Control	10 ppm CuSO <sub>4</sub>	10 ppm Availa Cu	20 ppm CuSO <sub>4</sub>	20 ppm Availa Cu		Control vs. Cu	10 ppm vs. 20 ppm	CuSO <sub>4</sub> vs. Availa Cu at 10 ppm	CuSO <sub>4</sub> vs. Availa Cu at 20 ppm	
	◯moles of glycerol released g tissue <sup>-1</sup> h <sup>-1</sup>										
Basal lipolysis	0.59	0.74	0.75	0.79	0.81	0.08	0.06	0.21	0.31	0.17	
Epinephrine stimulated lipolysis	1.34	1.59	1.60	1.65	1.72	0.11	0.04	0.27	0.28	0.16	