Effects of dietary copper supplementation on performance, carcass characteristics, and lipolytic rate of beef steers fed ractopamine hydrochloride

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INTRODUCTION
Nearly 85% of fed cattle in the United States receive a dietary beta agonist (Samuelson et al., 2016). Beta agonists activate intracellular signaling pathways to increase muscle accretion and lipid mobilization by the animal (Mersmann, 1998). The essential trace mineral Cu appears to regulate the beta agonist signaling pathway (Krishnamoorthy et al., 2016), and may affect mitochondrial uptake of fatty acids, necessary for beta oxidation (Song et al., 2012; Lei et al., 2017). Messersmith (2021) discovered rate of gain and lipolytic rate were dependent upon Cu supplementation in beta agonist-fed steers, corroborating this connection between Cu and lipid metabolism. However, how cattle of different initial liver Cu status respond to dietary Cu concentration has not been studied. Therefore, the objective of this study was to determine the effects of dietary Cu treatment on performance, carcass characteristics, and in vitro lipolytic rate of steers fed a beta agonist. A range in liver Cu concentrations (3 to 370 mg Cu/kg dry matter [DM]) was established in a previous study. Conducted as a randomized complete design, steers were stratified by initial liver Cu concentrations and body weight (BW) into pens (n = 5 or 6 steers per pen) equipped with GrowSafe bunks (GrowSafe Systems Ltd, Airdrie, AB, Canada). Cattle were randomly assigned to supplemental Cu treatment including 0, 5, 10, or 15 mg Cu/kg DM from bis-glycinate Cu (Plexomin Cu; Phytobiotics, Cary, NC; Cu0, Cu5, Cu10, Cu15, respectively). All steers were fed 300 mg per steer per day of ractopamine hydrochloride (Optaflexx; Elanco Animal Health, Greenfield, IN) in a dry-rolled corn-based finishing diet (Table 1) and harvested on day 32 at a commercial abattoir (National Beef, Tama, IA).

MATERIALS AND METHODS
The Iowa State University Institutional Animal Care and Use Committee (log number: IACUC-19-316) approved all procedures and protocols utilized in this study. Sixty-four Angus-cross steers (603 ± 36 kg) were utilized to test the effects of liver Cu concentration within Cu supplementation treatment on performance, carcass characteristics, and in vitro lipolytic rate of steers fed a beta agonist. A range in liver Cu concentrations (3 to 370 mg Cu/kg dry matter [DM]) was established in a previous study. Conducted as a randomized complete design, steers were stratified by initial liver Cu concentrations and body weight (BW) into pens (n = 5 or 6 steers per pen) equipped with GrowSafe bunks (GrowSafe Systems Ltd, Airdrie, AB, Canada). Cattle were randomly assigned to supplemental Cu treatment including 0, 5, 10, or 15 mg Cu/kg DM from bis-glycinate Cu (Plexomin Cu; Phytobiotics, Cary, NC; Cu0, Cu5, Cu10, Cu15, respectively). All steers were fed 300 mg per steer per day of ractopamine hydrochloride (Optaflexx; Elanco Animal Health, Greenfield, IN) in a dry-rolled corn-based finishing diet (Table 1) and harvested on day 32 at a commercial abattoir (National Beef, Tama, IA). Total mixed ration (TMR) samples were collected weekly, dried in a forced air oven, and ground via methods described by Pogge and Hansen (2013). Samples were composited by treatment and nutrient analysis conducted by Dairyland Laboratories (Arcadia, WI). Weights and blood
were collected on day 0 and 31 following a 16-h feed and water restriction. Blood was collected from all steers (n = 16 per treatment) via jugular venipuncture in vacuum capped tubes (Becton Dickerson, Rutherford, NJ) for serum and plasma collection. Serum nonesterified fatty acid (NEFA) concentrations were analyzed using a commercial kit (Wako Pure Chemical Industries, Ltd, Chuo-Ku, Osaka, Japan) with an intra-assay and inter-assay coefficient of variation of 3.2% and 10.7%, respectively.

Liver biopsies were conducted on day −16/−15 and 25/26 (n = 16 per treatment) and adipose biopsies on day 12/13 (n = 8 steers per treatment) following adapted procedures from Engle and Spears (2000) and Koltes and Spurlock (2011), respectively. Half of the steers were biopsied on each of the consecutive days. In vitro lipolytic rate was measured from adipose tissue via methods adapted from Pothoven et al. (1975) and released glycerol was analyzed via gas chromatography-mass spectrometry (GC-MS; Agilent Technologies Model 6890 GC coupled to Model 5975 MS) at the W. M. Keck Metabolomics Research Laboratory (Iowa State University, Ames, IA). Trace mineral was analyzed via inductively coupled plasma optical emission spectrometry (Optima 7000 DV; Perkin Elmer, Waltham, MA) following the procedures of Pogge and Hansen (2013).

Data were analyzed via Proc Mixed of SAS (SAS Institute, Cary, NC) with the fixed effect of Cu treatment and random effect of steer. Initial liver Cu concentration × treatment was utilized as a covariate in analysis excluding analysis of initial liver Cu concentrations. Initial BW served as a covariate for performance and carcass data while initial NEFA and trace mineral concentrations were covariates to the respective parameters. Contrast statements were formed to test linear, quadratic, and cubic effects of Cu supplementation. Correlations between initial liver Cu concentrations and final BW were tested via Proc Corr (SAS Institute). Cook’s D was utilized to test for outliers.

**RESULTS AND DISCUSSION**

Although beta agonists are well utilized in the beef industry (Samuelson et al., 2016), few studies have examined the effects of trace mineral status or supplementation on beta agonist-induced performance. Table 2 shows the results of the study.

### Table 1. Diet composition*

| Ingredient         | % of diet DM |
|--------------------|--------------|
| Dry-rolled corn    | 62.0         |
| MDGS†              | 20.0         |
| Bromegrass hay     | 8.0          |
| DDGS‡              | 8.05         |
| Limestone          | 1.5          |
| Salt               | 0.31         |
| Mineral and vitamin premix† | 0.125 |
| Rumensin           | 0.015        |

#### Analyzed composition
- Cu0 TMR was conducted by Dairyland Laboratories (Arcadia, WI).
- Modified distillers grains with solubles.
- Dried distillers grains with solubles.
- With the exception of Cu, trace minerals were supplemented at NASEM (2016) recommendations for Co, I, Mn, Se, and Zn. Inorganic sources were used for Co, I, and Se. Plexomin Mn and Plexomin Zn were fed in addition to the 0, 5, 10, or 15 mg Cu/kg DM from bis-glycinate Cu (Plexomin Cu; Phytobiotics, Cary, NC).
- Analyzed values for trace minerals represent Cu0 concentrations measured by inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV; Perkin Elmer, Waltham, MA).

### Table 2. Performance is influenced by Cu supplementation in beta agonist-fed steers

| Treatments*        | Cu0 | Cu5 | Cu10 | Cu15 | SEM | L  | Q  | C   |
|--------------------|-----|-----|------|------|-----|----|----|-----|
| Steers (n)‡         | 16  | 15  | 15   | 16   |     |    |    |     |
| Day 0, weights, kg | 593 | 611 | 605  | 607  | 8.9 | 0.03 | 0.37 | 0.20 |
| Day 31, weights, kg| 674 | 671 | 661  | 675  | 4.3 | 0.86 | 0.11 | 0.02 |
| Overall, ADG, kg   | 2.26| 2.16| 1.83 | 2.28 | 0.139 | 0.86 | 0.10 | 0.02 |
| Overall, DMI, kg   | 12.5| 12.6| 13.4 | 12.7 | 0.27 | 0.42 | 0.86 | 0.36 |
| G:F                | 0.18| 0.17| 0.14 | 0.18 | 0.011 | 0.97 | 0.07 | 0.05 |
| Carcass-adjusted‡  |     |     |      |      | 0.63 | 0.03 | 0.34 |     |
| Final, BW, kg      | 675 | 671 | 660  | 675  | 4.2 | 0.63 | 0.03 | 0.34 |
| Final, ADG, kg     | 2.20| 2.07| 1.73 | 2.21 | 0.131 | 0.64 | 0.02 | 0.32 |
| G:F                | 0.18| 0.17| 0.14 | 0.18 | 0.010 | 0.92 | 0.02 | 0.51 |

*Steers were fed 0, 5, 10, or 15 mg Cu/kg DM from bis-glycinate Cu (Plexomin Cu; Phytobiotics, Cary, NC) for Cu0, Cu5, Cu10, or Cu15, respectively. All cattle received 300 mg per steer per day of ractopamine hydrochloride (Optaflexx; Elanco Animal Health, Greenfield, IN).
†Contrast statements were formed to test linear (L), quadratic (Q), and cubic (C) effects of Cu supplementation.
‡Initial BW and initial liver Cu concentration × treatment served as covariates in analysis.
§Carcass-adjusted data were calculated using treatment averages for dressing percentage.
performance. Increasing supplemental Zn has been shown to linearly improve performance of steers (Genther-Schroeder et al., 2016) and Cu supplementation improved feed conversion in pigs (Feldpausch et al., 2015) receiving a beta agonist. Furthermore, Messersmith (2021) found beta agonist growth was dependent on Cu supplementation in finishing steers.

In the present study a linear increase in day 0 BW (Table 2; $P = 0.03$) was observed with Cu supplementation. Considering Cu treatments had not been administered and cattle were stratified to treatment by initial liver Cu concentrations, this effect appears the result of the covariate initial liver Cu concentration $\times$ treatment as raw treatment means do not depict this linear response. Day 0 BW was utilized as a covariate in subsequent performance and carcass data analysis to mediate any differences in initial BW due to Cu treatment. A cubic effect ($P \leq 0.05$) was observed for day 31 BW, overall average daily gain (ADG), and feed efficiency (G:F) in which Cu10 steers are lighter, gain less, and are less efficient than Cu0, Cu5, and Cu15. This contrasts the findings of Messersmith (2021) where steers supplemented 10 mg Cu/kg DM had greatest performance in comparison to those supplemented 0 or 20 mg Cu/kg DM from a Cu amino acid complex. Day 31 BW was positively correlated with initial liver Cu concentrations within Cu0 ($r = 0.60; P = 0.01$), though not correlated when grouping Cu5, Cu10, and Cu15 data ($r = -0.20; P = 0.18$). This suggests adequate initial liver Cu concentrations are important to performance.

### Figure 1.

Effects of increasing supplemental Cu in the diet of steers fed a beta agonist on liver and plasma Cu concentrations. Steers were supplemented 0, 5, 10, or 15 mg Cu/kg DM (Cu0, Cu5, Cu10, Cu15, respectively) from bis-glycinate Cu (Plexomin Cu; Phytobiotics, Cary, NC) and all cattle were fed 300 mg per steer per day of ractopamine hydrochloride (Optaflexx; Elanco Animal Health, Greenfield, IN). Liver biopsies were conducted on day $-16/-15$ and $25/26$ and plasma collected on day 0 and 31 for initial and final values. Polynomial contrast statements compared linear (L), quadratic (Q), and cubic (C) effects of Cu supplementation. Initial liver Cu concentration $\times$ treatment was a covariate in all analysis excluding initial liver Cu concentration analysis. Initial liver and plasma Cu concentrations served as covariates in final liver and plasma Cu analysis. (A) By design, Cu supplementation did not affect initial liver Cu concentrations ($P \geq 0.87$). Final liver Cu concentrations linearly increased with increasing Cu supplementation ($P < 0.01$) and a tendency for a cubic effect ($P = 0.06$) was observed. (B) No linear, quadratic, or cubic effects of Cu supplementation were observed for initial or final plasma Cu concentrations ($P \geq 0.21$).

### Table 3. Effects of Cu supplementation on carcass characteristics in beta agonist-fed steers

| Treatments* | SEM | L  | Q  | C  |
|-------------|-----|----|----|----|
| Cu0         | 2.6 | 0.38 | 0.37 | 0.49 |
| Cu5         | 3.6 | 0.44 | 0.35 | 0.002 |
| Cu10        | 4.0 | 0.48 | 0.61 | 0.99 |
| Cu15        | 3.9 | 0.44 | 0.90 | 0.41 |

*Steers were fed 0, 5, 10, or 15 mg Cu/kg DM from bis-glycinate Cu (Plexomin Cu; Phytobiotics, Cary, NC) for Cu0, Cu5, Cu10, or Cu15, respectively. All cattle received 300 mg per steer per day of ractopamine hydrochloride (Optaflexx; Elanco Animal Health, Greenfield, IN).

†Contrast statements were formed to test linear (L), quadratic (Q), and cubic (C) effects of Cu supplementation.

‡Initial BW and initial liver Cu concentration $\times$ treatment served as covariates in analysis.

‖Marbling scores: slight = 300, small = 400, modest = 500, moderate = 600.
Dietary copper during high growth rates

outcomes when no supplemental Cu is offered. No polynomial effects ($P \geq 0.36$) were observed for DM intake. However, carcass-adjusted final BW, ADG, and G:F observed a quadratic response ($P \leq 0.03$) to Cu supplementation in which Cu10 had lesser performance than Cu0, Cu5, and Cu15, similar to the cubic responses in live performance data.

By study design, initial liver (Figure 1A) and plasma (Figure 1B) Cu concentrations were similar among treatments ($P \geq 0.44$). However, final liver Cu concentrations linearly increased with increasing Cu supplementation ($P < 0.01$) with a tendency ($P = 0.06$) for a cubic effect. Copper supplementation drastically improved liver Cu concentrations during 31-d study (−0.9%, +33.3%, +52.0%, and +48.1% in liver Cu concentrations for Cu0, Cu5, Cu10, and Cu15, respectively). Day 31 plasma Cu concentrations were not affected ($P \geq 0.21$) by Cu supplementation. All final trace mineral treatment means were within the adequacy range proposed by Kincaid (2000; liver: 125 to 600 mg Cu/kg DM; plasma: >0.7 mg/L); but varied greatly (liver: 4 to 458 mg/kg DM; plasma: 0.4 to 1.8 mg/L).

A cubic effect (Table 3; $P = 0.002$) for dressing percentage was observed in which Cu10 had greater dress than all other treatments. No additional polynomial effects ($P \geq 0.27$) were observed for carcass characteristics including: hot carcass weight, ribeye area, back fat, kidney pelvic heart fat, marbling, or yield grade. Furthermore, no effects of Cu supplementation were observed on day 0 or 31 NEFA concentrations and basal lipolytic rate (Table 4; $P \geq 0.14$). However, a tendency for a quadratic response to Cu supplementation was observed in stimulated lipolytic rate ($P = 0.06$) in which Cu15 was lesser than all other treatments; though Cu0, Cu5, and Cu10 were not different from one another. Copper has been linked to the regulation of lipolysis through phosphodiesterase, an inhibitor of the beta agonist pathway (Krishnamoorthy et al., 2016) and the uptake of fatty acids into the mitochondria for beta oxidation (Song et al., 2012; Lei et al., 2017).

Together these data provide insights into how performance of beta agonist-fed steers is influenced by both liver and supplemental Cu concentrations. These data suggest adequate liver Cu concentrations are necessary for acceptable growth rates when supplemental Cu is restricted. However, supplemental Cu, regardless of concentration tested, appears to ablate the necessity of adequate liver Cu stores. Therefore, it appears late-stage finishing steers have a low requirement for dietary Cu even if minimal Cu supplementation is provided.

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Conflict of interest statement. The authors declare no conflicts of interest.

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**Table 4. Effect of Cu supplementation on NEFA and in vitro lipolytic rate**

| Treatments | Cu0 | Cu5 | Cu10 | Cu15 | SEM | L | Q | C |
|------------|-----|-----|------|------|-----|---|---|---|
| NEFA, mEQ/L |     |     |      |      |     |   |   |   |
| Day 0      | 329 | 270 | 279  | 302  | 32.3| 0.75 | 0.57 | 0.90 |
| Day 31     | 229 | 254 | 303  | 216  | 24.2| 0.79 | 0.14 | 0.90 |
| Lipolytic rate, µmol glycerol/g tissue/h |     |     |      |      |     |   |   |   |
| Basal      | 0.29| 0.23| 0.27 | 0.28 | 0.041| 0.93 | 0.20 | 0.25 |
| Stimulated | 0.25| 0.25| 0.22 | 0.16 | 0.025| 0.75 | 0.06 | 0.18 |

*Steers were fed 0, 5, 10, or 15 mg Cu/kg DM from bis-glycinate Cu (Plexomin Cu; Phytobiotics, Cary, NC) for Cu0, Cu5, Cu10, or Cu15, respectively. All cattle received 300 mg per steer per day of ractopamine hydrochloride (Optaflexx; Elanco Animal Health, Greenfield, IN).

†Contrast statements were formed to test linear (L), quadratic (Q), and cubic (C) effects of Cu supplementation.

‡Initial values and initial liver Cu × treatment served as covariates in analysis.

‖Media was stimulated with epinephrine.
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