Effects of increasing supplemental dietary Zn concentration on growth performance and carcass characteristics in finishing steers fed ractopamine hydrochloride

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ABSTRACT: Angus-cross steers ($n = 288$; 427 ± 0.4 kg) were utilized in a finishing study to evaluate the influence of increasing dietary Zn concentration on growth performance and carcass characteristics of steers fed ractopamine hydrochloride (RAC). In a randomized complete block design, steers were blocked by weight (6 steers/pen) and fed a dry-rolled corn-based diet for 79 d containing no supplemental Zn (CON; $n = 8$), 60 mg Zn/kg from ZnSO_4 and no supplemental Zn-amino acid complex (ZnAA; ZnAA0; $n = 8$) or ZnAA0 diet supplemented with 60 (ZnAA60; $n = 8$), 90 (ZnAA90; $n = 7$), 120 (ZnAA120; $n = 8$), or 150 (ZnAA150; $n = 8$) mg Zn/kg DM from ZnAA. Thirty-one days prior to harvest (day 48 of study) all steers began receiving RAC at $300 \text{ mg} \cdot \text{steer}^{-1} \cdot \text{d}^{-1}$. This study was organized as 2 groups (GRP) of steers and groups were staggered so that GRP1 started and ended 2 wk before GRP2. Pen was the experimental unit, and the statistical model included the fixed effects of treatment and block nested within GRP. Three a priori single degree of freedom contrasts were

developed: linear and quadratic effects of ZnAA supplementation (ZnAA0, ZnAA60, ZnAA90, ZnAA120, and ZnAA150), and CON vs. Zn (CON vs. ZnAA0, ZnAA60, ZnAA90, ZnAA120, and ZnAA150). Dietary Zn concentration did not affect growth performance prior to RAC supplementation ($P \geq 0.17$). During the RAC-period ADG and DMI were not affected by dietary Zn ($P \geq 0.16$), while there was a linear effect of dietary Zn supplementation to decrease G:F ($P = 0.04$). Marbling scores were greatest in CON steers ($P = 0.03$). Liver Cu (day 45 and 80) and meat Cu (harvest) concentrations were greater in CON steers relative to Zn-supplemented steers ($P \leq 0.05$), and plasma Zn linearly increased as dietary Zn increased ($P = 0.007$). Warner-Bratzler shear force was not different among treatments ($P \geq 0.25$), and meat total collagen was quadratically affected by dietary Zn supplementation ($P \leq 0.002$) where ZnAA0 was greatest. Overall, there was no effect of dietary Zn concentration on growth performance of RAC-supplemented steers in this study.

Key words: beef, cattle, zinc

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INTRODUCTION

Zinc (Zn) is essential for growth in animals. Research data from large pen feedlot studies (Larson and Branine, 2015) and previous university studies (Genther-Schroeder et al., 2016a,

2016b) have indicated that feeding Zn beyond the requirement of finishing steers may result in additional hot carcass weight (HCW) when cattle are fed ractopamine hydrochloride (RAC). However, this response may be related to Zn source and concentration, as others have not reported a relationship between Zn supplementation and RAC (Bohrer et al., 2014; Edenburn et al., 2016). Zinc is a critical component of many proteins and enzymes, including matrix metalloproteinases

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(Nagase et al., 2006), which are responsible for breakdown of the extracellular matrix. Turnover of the extracellular matrix is essential for muscle and marbling development, as well as ultimate meat tenderness (Purslow et al., 2012; Velleman, 2012). Zinc supplementation may result in increased activity of these enzymes, which could impact product quality and tenderness.

Previously, a linear increase in the growth of cattle supplemented with RAC was observed as Zn-amino acid complex (ZnAA) supplementation increased from 0 to 90 mg Zn/kg DM (Genther-Schroeder et al., 2016a). While total dietary Zn in the control diet was approximately 3-fold the NRC (2000) recommendation for Zn (88 mg Zn/kg DM), an improvement in ADG and feed efficiency was observed as ZnAA increased in the diet. The linear nature of this response indicated that even at 90 mg Zn/kg DM from ZnAA there was no plateau in live performance and HCW, suggesting that greater concentrations of ZnAA could further improve performance. It is unknown if performance would continue to improve as Zn-AA supplementation in the diet increased and to what level this response would achieve a plateau.

The objective was to determine how additional dietary Zn would affect growth response and ultimate meat quality of finishing steers. The hypothesis is that providing additional dietary Zn supplemented as ZnAA, and a β -agonist, to finishing cattle would increase growth response, but would plateau at high dietary Zn concentrations, and would improve meat quality.

MATERIALS AND METHODS

All procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (log number 11-15-8121-B).

Two hundred eighty-eight Angus-type steers (427 ± 0.4 kg) were utilized in a finishing study to evaluate the influence of increasing dietary Zn concentration on growth performance and carcass characteristics of steers fed RAC (Optaflexx; Elanco Animal Health, Greenfield, IN). Steers were purchased from 2 sources and sources were balanced among pens. Steers were housed in pens of 6 with continuous access to shade. All steers were fed the same basal finishing-type diet (Table 1). To accommodate the size of the experiment, one group (GRP) of 144 steers (GRP 1) was started 2 wk before the second group (GRP 2) of steers, and were blocked by BW to pens with 6 steers each, and pens within blocks were randomly assigned to

dietary treatments at the beginning of the finishing period. Dietary treatments were CON) no supplemental Zn (total diet analyzed 30 mg Zn/kg DM, $n = 8$ pens), ZnAA0) 60 ppm supplemental Zn from ZnSO₄ (analyzed 95 mg Zn/kg DM, $n = 8$ pens), ZnAA60) 60 ppm supplemental Zn from ZnSO₄ + 60 ppm supplemental Zn from ZnAA (analyzed 152 mg Zn/kg DM, $n = 8$ pens), ZnAA90) 60 ppm supplemental Zn from ZnSO₄ + 90 ppm supplemental Zn from ZnAA (analyzed 188 mg Zn/kg DM, $n = 7$ pens), ZnAA120) 60 ppm supplemental Zn from ZnSO₄ + 120 ppm supplemental Zn from ZnAA (analyzed 209 mg Zn/kg DM, $n = 8$ pens), and ZnAA150) 60 ppm supplemental Zn from ZnSO₄ + 150 ppm supplemental Zn from ZnAA (analyzed 230 mg Zn/kg DM, $n = 8$ pens). Steers had ad libitum access to water and were fed for approximately 3% feed refusal as previously described (Genther and Hansen, 2014).

All steers were implanted with Component TE-IS with Tylan (Elanco Animal Health) on day 0 for each group, and were vaccinated against bovine rhinotracheitis, virus diarrhea, parainfluenza-3, respiratory syncytial virus, *Mannheimia haemolytica*,

Table 1. Composition of control (0 mg of supplemental Zn) diet

Ingredient	% of diet DM
Dry rolled corn	62.0
Modified corn distiller's grains with solubles	25.0
Bromegrass hay	8.0
Dried corn distiller's grains with solubles	2.95
Limestone	1.50
Salt	0.31
Vitamin A premix ¹	0.11
Trace mineral premix ²	0.10
Rumensin90 ³	0.01
Calculated composition ⁴	
CP, %	13.6
NDF, %	18.4
NE _g , Mcal/kg	1.39
Ether extract, %	4.85
Analyzed composition ⁵ , mg/kg DM	
Cu	18.8
Fe	99.7
Zn	30

¹Vitamin A premix contained 4,400,000 IU/kg.

²Provided per kg of diet: 60 mg Zn; 48 mg Mn; 0.75 mg I; 0.24 mg Se; 17.6 mg Cu; and 0.38 mg Co (all inorganic sources); concentrations from Vasconcelos and Galyean (2007).

³Provided at 27 g/909.1 kg of diet (Elanco Animal Health).

⁴Composition was calculated using values from the NRC (2000).

⁵Trace mineral analysis was conducted using inductively coupled plasma optical emission spectroscopy as described by Pogge et al. (2014).

Pasteurella multocida (Vista once SQ, Merck Animal Health, Summit, NJ), and *Clostridium chauvoei-septicum-novyi-sordellii-perfringens* Types C & D, bacterin-toxoid (Vision 7, Merck Animal Health).

Steers were weighed on days -1, 0, 28, 47, and 48. On day 48, all steers began receiving RAC at 300 mg·steer⁻¹·day⁻¹. Liver biopsy samples were collected on day 45 using the methods of [Engle and Spears \(2000\)](#) from one randomly-selected steer per pen ($n = 48$). Steers were weighed on day 62 (14 d RAC) and 78 and 79. After 31 d of RAC supplementation, all steers within a GRP were shipped to Iowa Premium Beef (Tama, IA) on day 79, to be harvested on day 80. A liver sample from the left lobe was collected from the 48 sampler steers that had previously been selected for biopsy. Hot carcass weights were collected immediately after harvest. Carcass data were collected after a 48 h chill, according to USDA standards, including ribeye area (REA), 12th rib back fat (BF), KPH, and marbling score (MS). Marbling scores were used to determine quality grade (QG), and yield grade (YG) was calculated according to the [USDA \(1997\)](#). Carcass-adjusted performance variables were calculated by dividing individual HCW with the average dressing percentage (64.3%). A 4% pencil shrink was applied to all live BW measures as well as in the calculation of ADG and G:F.

Sample Collection

Jugular blood samples were collected via venipuncture on day 0, 48, 62, and 79 from one randomly selected steer per pen into tubes containing either K₂EDTA for trace mineral (TM) analysis, or sodium heparin for plasma cAMP concentrations. A 5 cm rib section from the 12th rib was collected after a 48 h chill from 2 steers per pen from GRP 2. A liver section from the left lobe was also collected from these steers at harvest to determine TM status. Rib sections were transported back to the laboratory on ice, and were immediately de-boned, and a 2.5 cm steak was cut, and vacuum packaged, and then aged in a cooler at 2°C for 14 d, after which they were frozen at -20°C until analyzed for Warner-Bratzler shear force. Two additional, approximately 5 cm³ sections, were collected and stored at -20°C until further analysis of TM concentration and soluble and insoluble collagen.

Total mixed ration (TMR) samples were collected weekly and dried in a forced-air oven at 70°C for 48 h to determine DM content. DMI was calculated using as-fed intakes corrected for weekly

TMR DM. Feed efficiency, measured as gain:feed (G:F) was calculated using total gain and total DMI between weighing intervals for each pen. Diet samples were ground through a 2 mm sieve in a Wiley mill (Thomas Scientific, Swedesboro, NJ) and composited within treatment by month for TM analysis.

Plasma and Tissue Analyses

Liver biopsy samples, liver sections collected at harvest, and meat samples were dried in a forced-air oven at 70°C for 72 h, and digested using TM-grade nitric acid before mineral analysis (MARSXpress; CEMS, Matthews, NC) as described by [Richter et al. \(2012\)](#). Trace mineral analysis of feed, tissues, and plasma was conducted using inductively coupled plasma optical emission spectroscopy (PerkinElmer, Waltham, MA). Tissue and plasma TM analysis was completed as described by [Pogge and Hansen \(2013\)](#). Feed TM analysis was completed as described by [Pogge et al. \(2014\)](#).

Plasma samples for cAMP analysis (as a measurement of the β -adrenergic cascade) were purified by the addition of ice-cold ethanol, followed by centrifugation at $1,500 \times g$. The supernatant was dried under a stream of N gas and then resuspended in a buffer provided by the manufacturer listed below. Sample cAMP concentration was analyzed using a commercial ELISA kit (Cayman Chemical, Ann Arbor, MI, Catalog #501040).

Warner-bratzler Shear Force and Soluble and Insoluble Collagen

Samples for soluble and total collagen were homogenized in a Waring blender with liquid nitrogen prior to analysis. Total soluble and insoluble collagen content was determined ($n = 48$) using the methods of [Hill \(1966\)](#) and [Bergman and Loxley \(1963\)](#). Hydroxyproline content was converted to collagen (μg collagen/g of sample) by multiplying the hydroxyproline concentration of supernatants by 7.52 and residuals by 7.25 ([Cross et al., 1973](#)).

Steaks for Warner-Bratzler shear force analysis were thawed at 2°C for 48 h prior to cooking. Steaks were cooked on a clam-shell grill to an internal temperature of 69°C, individually monitored using thermocouples. Steaks were cooled to room temperature prior to collecting four core samples from each steak (approximately 1.27 cm, parallel to the direction of the muscle fibers). The Instron Universal Testing Machine (Model 5566, Instron Corporations, Norwood, MA) was used to

determine the peak force (kg of shear force) required to shear perpendicularly through the 0.63 cm diameter cores with 10 kN of load cell and a head speed of 200 mm/min. The four measurements per steak were averaged into a single value for analysis.

Statistical Analysis

Data were analyzed using the mixed procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC) as a randomized complete block design. Pen was the experimental unit for all data. One entire pen (ZnAA90) was removed as an outlier from both Pre-RAC and RAC periods for poor performance caused by illness unrelated to treatment. Plasma analytes were analyzed as repeated measures, and the subject for the repeated statement was pen nested within dietary treatment and group. Three a priori individual degree of freedom contrasts were developed using the IML procedure of SAS 9.3; the linear and quadratic effects of formulated Zn-AA (ZnAA0, ZnAA60, ZnAA90, ZnAA120, and ZnAA150), and Con vs. Zn: which compared the CON treatment to all other treatments. Correlations were performed using the CORR procedure in SAS 9.3. Quality grade distributions were evaluated using the GLIMIX procedure in SAS 9.3. Data were checked for normalcy and homogeneity of variance, and transformed when necessary, but only back-transformed data are reported. Outliers were determined using Cook's D statistic and removed if Cook's $D > 0.5$. One steer was determined to be an outlier for day 48 liver biopsy TM and data were

removed. Data reported are least-squared means $\pm$ SEM. Significance was declared at $P \leq 0.05$ and tendencies were declared from $P = 0.06$ to 0.10.

RESULTS

Pre-RAC Period Steer Growth Performance

Dietary Zn concentration did not influence ADG, DMI, G:F, or BW ($P \geq 0.17$) during the 48 d prior to RAC supplementation (Table 2).

RAC Period Steer Growth Performance

There was no difference among Zn treatments in BW after 14 or 31 d of RAC supplementation ($P \geq 0.54$; Table 3). Dietary Zn concentration did not affect ADG, DMI or G:F ($P \geq 0.18$) during the first 14 d, or the final 17 d of RAC supplementation ($P \geq 0.21$). Across the entire RAC period (day 48 through 79), there were no differences due to dietary Zn concentration on ADG ($P \geq 0.16$) or DMI ($P \geq 0.48$); however, there was a linear decrease in G:F as dietary Zn concentration increased ($P = 0.04$). Over the entire feeding period (day 0 through 79), ADG ($P \geq 0.62$), DMI ($P \geq 0.35$), and G:F ($P \geq 0.16$) were not different due to treatment.

There was no effect of dietary Zn on HCW ($P \geq 0.55$; Table 4). There was a tendency for a quadratic effect of ZnAA supplementation ($P = 0.09$) on dressing percentage likely because of slightly greater dressing percentage in ZnAA0. Marbling scores were greater in CON steers than steers that

Table 2. The effect of supplemental Zn on finishing steer performance during the pre- RAC period (day 0 through 47)¹

Variable	Treatments ²						SEM	Contrast P -values ³		
	CON	ZnAA0	ZnAA60	ZnAA90	ZnAA120	ZnAA150		LZn	QZn	CON vs. Zn
Pens	8	8	8	7	8	8				
Initial BW, kg	427	427	427	427	426	427	0.6	0.51	0.54	0.90
Pre-RAC period (day 0 through 47)										
Day 48 BW, kg	522	517	521	523	516	521	2.6	0.63	0.44	0.44
Day 0 to 47										
ADG, kg/d	1.98	1.91	1.96	2.01	1.88	1.96	0.054	0.80	0.50	0.51
DMI, kg/d	11.4	10.9	11.1	11.2	11.1	11.2	0.20	0.30	0.66	0.17
G:F	0.174	0.175	0.176	0.180	0.171	0.175	0.0031	0.67	0.56	0.72

¹A 4% pencil shrink was applied to all live BW measures as well as in the calculation of ADG and G:F.

²Treatments: a dry-rolled corn-based diet supplemented with 0 mg Zn/kg from ZnSO₄ and no supplemental ZnAA (CON; analyzed 30 mg Zn/kg DM), 60 mg Zn/kg from ZnSO₄ and no supplemental ZnAA (ZnAA0; analyzed 95 mg Zn/kg DM) or ZnAA0 diet supplemented with 60 (ZnAA60; analyzed 152 mg Zn/kg DM), 90 (ZnAA90; analyzed 188 mg Zn/kg DM), 120 (ZnAA120; analyzed 209 mg Zn/kg DM) or 150 (ZnAA150; analyzed 230 mg Zn/kg DM) mg Zn/kg DM from ZnAA.

³Contrasts: LZn = the linear effect of ZnAA; QZn = the quadratic effect of ZnAA. CON vs. Zn = CON vs. ZnAA0, ZnAA60, ZnAA90, ZnAA120, and ZnAA150.

Table 3. The effect of supplemental Zn for 79 d and ractopamine hydrochloride (RAC) supplementation (300 mg·steer⁻¹d⁻¹) for 31 d on finishing steer performance during the first 14 d of the RAC period (day 48 through 61) and the final 17 d of the RAC period (day 62 through 79)¹

Variable	Treatments ²						SEM	Contrast <i>P</i> -values ³		
	CON	ZnAA0	ZnAA60	ZnAA90	ZnAA120	ZnAA150		LZn	QZn	CON vs. Zn
Pens	8	8	8	7	8	8				
RAC period										
Overall (day 48 through 79)										
ADG, kg/d	2.04	2.16	2.13	2.09	2.07	2.09	0.049	0.16	0.67	0.24
DMI, kg/d	11.9	11.7	11.9	12.0	11.9	11.9	0.22	0.48	0.50	0.96
G:F	0.172	0.185	0.178	0.173	0.174	0.176	0.0037	0.04	0.25	0.16
Day 62 BW, kg	555	552	554	557	550	554	3.3	0.95	0.54	0.73
Day 48 to 62										
ADG, kg/d	2.31	2.46	2.37	2.40	2.40	2.39	0.137	0.76	0.78	0.54
DMI, kg/d	11.7	11.4	11.7	11.8	11.7	11.8	0.25	0.26	0.72	0.92
G:F	0.203	0.221	0.201	0.204	0.204	0.202	0.0096	0.18	0.38	0.73
Day 79 BW, kg	585	585	587	588	581	585	3.1	0.72	0.59	0.90
Day 63 to 79										
ADG, kg/d	2.21	2.33	2.34	2.23	2.17	2.24	0.087	0.21	0.91	0.60
DMI, kg/d	12.1	11.9	12.2	12.2	12.0	12.0	0.21	0.87	0.30	0.99
G:F	0.151	0.162	0.159	0.150	0.150	0.154	0.0071	0.26	0.59	0.64
Overall (day 0 through 79)										
ADG, kg/d	2.01	2.01	2.02	2.04	1.95	2.01	0.039	0.65	0.62	0.99
DMI, kg/d	11.7	11.3	11.5	11.6	11.5	11.6	0.19	0.35	0.54	0.46
G:F	0.171	0.177	0.174	0.178	0.172	0.172	0.0025	0.17	0.72	0.16

¹A 4% pencil shrink was applied to all live BW measures as well as in the calculation of ADG and G:F.

²Treatments: a dry-rolled corn-based diet supplemented with 0 mg Zn/kg from ZnSO₄ and no supplemental ZnAA (CON; analyzed 30 mg Zn/kg DM), 60 mg Zn/kg from ZnSO₄ and no supplemental ZnAA (ZnAA0; analyzed 95 mg Zn/kg DM) or ZnAA0 diet supplemented with 60 (ZnAA60; analyzed 152 mg Zn/kg DM), 90 (ZnAA90; analyzed 188 mg Zn/kg DM), 120 (ZnAA120; analyzed 209 mg Zn/kg DM) or 150 (ZnAA150; analyzed 230 mg Zn/kg DM) mg Zn/kg DM from ZnAA.

³Contrasts: LZn = the linear effect of ZnAA; QZn = the quadratic effect of ZnAA. CON vs. Zn = CON vs. ZnAA0, ZnAA60, ZnAA90, ZnAA120, and ZnAA150.

received supplemental Zn ($P = 0.03$). Quality grade also tended ($P = 0.06$) to be greater in CON steers relative to Zn supplemented steers. Dietary Zn supplementation did not affect REA, BF, KPH, or YG ($P \geq 0.18$). Carcass adjusted final BW, ADG, and G:F over the entire feeding period were not influenced by dietary Zn concentration ($P \geq 0.28$).

Liver and Plasma Analysis

There was a tendency for a linear effect of supplemental ZnAA on plasma Cu concentrations ($P = 0.10$; Table 5) where plasma Cu concentration decreased as dietary Zn concentration increased, and CON steers had greater plasma Cu than steers supplemented with Zn ($P = 0.01$). Plasma Fe concentrations were unaffected by treatment ($P \geq 0.12$). Plasma Zn concentrations linearly increased ($P = 0.007$) as ZnAA supplementation increased. In addition, CON steers had lesser plasma Zn concentrations than Zn-supplemented steers ($P = 0.005$).

Plasma cAMP concentrations were greater in CON steers than Zn-supplemented steers ($P = 0.003$).

After 45 d, CON steers had greater liver Cu ($P = 0.01$) and lesser liver Zn concentrations ($P = 0.02$) than Zn-supplemented steers. Liver Fe concentrations ($P \geq 0.12$) were unaffected by dietary Zn concentrations.

Similar to the pre-RAC period, CON steers had greater liver Cu concentrations than Zn supplemented steers ($P = 0.003$). In contrast to the pre-RAC period, liver Zn concentrations assessed on tissues collected at harvest were unaffected by dietary Zn concentration ($P \geq 0.25$). Liver Fe concentrations were greater in CON steers than Zn-supplemented steers ($P = 0.03$).

Meat Cu concentrations were greater in CON steers than Zn-supplemented steers ($P = 0.02$; Table 6) and tended to be linearly increased as ZnAA supplementation increased ($P = 0.07$). There was a tendency for a quadratic effect of ZnAA supplementation on meat Fe concentrations ($P = 0.08$) where

Table 4. The effect of supplemental Zn for 79 d and ractopamine hydrochloride supplementation (300 mg·steer⁻¹·d⁻¹) for 31 d on finishing steer carcass performance

	Treatments ¹							Contrast <i>P</i> -values ²		
Variable	CON	ZnAA0	ZnAA60	ZnAA90	ZnAA120	ZnAA150	SEM	LZn	QZn	CON vs. Zn
Pens	8	8	8	7	8	8				
HCW, kg	376	377	375	378	373	376	2.2	0.55	0.89	0.87
Dress, %	64.2	64.7	64.1	64.0	64.4	64.2	0.21	0.09	0.09	0.66
Ribeye area, cm ²	81.4	82.6	82.1	81.3	81.1	82.0	0.77	0.30	0.38	0.61
Marbling score ³	431	419	411	422	425	417	4.9	0.59	0.85	0.03
Back fat, cm	1.48	1.38	1.42	1.42	1.49	1.47	0.059	0.18	0.96	0.49
KPH, %	2.09	2.11	2.09	2.10	2.13	2.05	0.053	0.67	0.64	0.85
Calculated YG	3.49	3.34	3.39	3.45	3.50	3.44	0.077	0.18	0.67	0.43
USDA Quality grade ⁴ , %										
Choice	89.0	88.8	83.0	77.1	87.2	81.0	5.36	0.42	0.46	0.36
Select	11.0	11.2	17.0	22.9	12.8	19.0	5.36	0.42	0.46	0.36
Carcass adjusted ⁵										
Final BW, kg	585	586	583	588	581	585	3.5	0.55	0.89	0.87
Overall ADG ⁶ , kg/d	2.00	2.02	1.98	2.03	1.96	1.99	0.043	0.57	0.87	0.99
G:F	0.170	0.178	0.170	0.177	0.173	0.171	0.0039	0.28	0.76	0.40
Overall DMI, kg	11.7	11.3	11.5	11.6	11.4	11.6	0.19	0.35	0.54	0.46

¹Treatments: a dry-rolled corn-based diet supplemented with 0 mg Zn/kg from ZnSO₄ and no supplemental ZnAA (CON; analyzed 30 mg Zn/kg DM), 60 mg Zn/kg from ZnSO₄ and no supplemental ZnAA (ZnAA0; analyzed 95 mg Zn/kg DM) or ZnAA0 diet supplemented with 60 (ZnAA60; analyzed 152 mg Zn/kg DM), 90 (ZnAA90; analyzed 188 mg Zn/kg DM), 120 (ZnAA120; analyzed 209 mg Zn/kg DM) or 150 (ZnAA150; analyzed 230 mg Zn/kg DM) mg Zn/kg DM from ZnAA.

²Contrasts: LZn = the linear effect of ZnAA; QZn = the quadratic effect of ZnAA. CON vs. Zn = CON vs. ZnAA0, ZnAA60, ZnAA90, ZnAA120, and ZnAA150.

³Marbling scores: small = 400; modest = 500; moderate = 600.

⁴Quality grades are based on percentages within treatment.

⁵Carcass-adjusted performance was calculated by dividing HCW with the average dressing percentage (64.3%).

⁶Overall ADG = ADG calculated from day 0 through the end of the experiment.

concentrations were similar between ZnAA0 and ZnAA60 calves, and increased from ZnAA90 to ZnAA120, and then decreased again in ZnAA150 calves.

There was no effect of dietary Zn concentration on steak WBSF ($P \geq 0.25$). Total collagen and insoluble collagen were affected quadratically by ZnAA supplementation ($P = 0.002$) where ZnAA0 steers had the greatest concentration and concentrations were least in the ZnAA60 steers, but increased steadily from ZnAA60 to ZnAA150. There was no effect of dietary Zn concentration on soluble collagen concentrations ($P \geq 0.17$). There was no correlation between meat TM concentrations and WBSF ($P \geq 0.34$) or collagen concentrations and WBSF ($P \geq 0.54$).

DISCUSSION

Previous work has demonstrated a synergy between ZnAA supplementation and RAC supplementation. In past work, we have noted that increasing ZnAA supplementation from 0 to 90 mg

Zn/kg DM in steers supplemented with RAC at 300 mg·steer⁻¹·day⁻¹ day linearly increased ADG (Genther-Schroeder et al., 2016a). A follow-up study reported that while 60 mg Zn/kg DM from ZnAA did not prevent the diminishing response to RAC typically seen as days on RAC increases from 28 to 42 d (Abney et al., 2007), ZnAA supplementation once again increased performance of yearlings fed RAC (Genther-Schroeder et al., 2016b). In this study, supplemental Zn from ZnSO₄ or ZnAA had minimal effects on growth performance in steers fed RAC at 300 mg·steer⁻¹·day⁻¹ day. It was hypothesized that ZnAA supplementation up to at least 90 mg Zn/kg would improve ADG during the RAC period, similar to previously reported results (Genther-Schroeder et al., 2016a). The calf-fed steers in this experiment were of different size and frame than the yearlings used in previous experiments; the steers began RAC at a lighter weight (approximately 25 to 36 kg lighter on average) and were harvested at a lighter weight (approximately 18 to 61 kg lighter on average) than steers in those

Table 5. The effect of supplemental Zn for 79 d and ractopamine hydrochloride supplementation (300 mg·steer⁻¹d⁻¹) for 31 d on finishing steer plasma and liver mineral and plasma cAMP¹

Variable	Treatments ²						SEM	Contrast <i>P</i> -values ³		
	CON	ZnAA0	ZnAA60	ZnAA90	ZnAA120	ZnAA150		LZn	QZn	CON vs. Zn
Pens	8	8	8	7	8	8				
Plasma mineral, ⁴ mg/L										
Cu	1.09	1.02	1.02	1.03	1.01	0.95	0.027	0.10	0.14	0.01
Fe	2.08	2.02	1.90	2.19	2.16	1.91	0.062	0.89	0.12	0.55
Zn	1.21	1.27	1.24	1.34	1.36	1.37	0.032	0.007	0.48	0.005
Plasma cAMP ⁵ , pmol/L	0.79	0.63	0.54	0.60	0.62	0.57	0.055	0.75	0.66	0.003
Liver mineral, mg/kg DM										
Day 45 ⁶										
Cu	346	285	277	296	261	241	26.7	0.26	0.38	0.01
Fe	173	177	185	185	194	160	11.9	0.64	0.12	0.61
Zn	96	113	122	124	115	119	8.5	0.71	0.47	0.02
Day 80 (collected at harvest) ⁷										
Cu	419	321	337	359	312	296	27.0	0.50	0.17	0.003
Fe	191	165	164	159	158	155	12.4	0.50	0.87	0.03
Zn	139	140	153	144	154	154	8.5	0.25	0.82	0.28

¹One randomly selected animal was sampled per pen.

²Treatments: a dry-rolled corn-based diet supplemented with 0 mg Zn/kg from ZnSO₄ and no supplemental ZnAA (CON; analyzed 30 mg Zn/kg DM), 60 mg Zn/kg from ZnSO₄ and no supplemental ZnAA (ZnAA0; analyzed 95 mg Zn/kg DM) or ZnAA0 diet supplemented with 60 (ZnAA60; analyzed 152 mg Zn/kg DM), 90 (ZnAA90; analyzed 188 mg Zn/kg DM), 120 (ZnAA120; analyzed 209 mg Zn/kg DM) or 150 (ZnAA150; analyzed 230 mg Zn/kg DM) mg Zn/kg DM from ZnAA.

³Contrasts: LZn = the linear effect of ZnAA; QZn = the quadratic effect of ZnAA. CON vs. Zn = CON vs. ZnAA0, ZnAA60, ZnAA90, ZnAA120, and ZnAA150.

⁴Based on repeated measures from day 0, 48, 62, and 79, treatment × day: *P* ≥ 0.72.

⁵cAMP = cyclic adenosine monophosphate; Based on repeated measures from day 48, 62, and 79, treatment × day *P* = 0.78.

⁶Liver samples were collected via biopsy (Engle and Spears, 2000).

⁷Liver samples were collected at abattoir postharvest.

previously mentioned experiments. Similarly, despite BF being very similar across studies indicating that steers reached a similar state of finish, the REA of the steers in this study were approximately 2.6 to 7.7 cm² smaller on average than the previous studies, though REA/cwt of carcass is similar. These differences in final weight, overall size and protein deposition of the steers may have influenced their response to both Zn and RAC, and led to differences in performance. Cattle of different composition or growth stage may respond differently to supplemental Zn. Heavier yearling cattle may have a greater performance response to β-agonist administration and a greater concentration of β-adrenergic receptors in skeletal muscle (Johnson et al., 2014) than younger, calf-fed cattle. The authors are unaware of any studies examining the Zn requirements of calf-fed vs. yearling feedlot cattle and further work is needed in this area.

Because many advances in cattle production have occurred since Zn requirements were initially established, including genetic advances and

technologies like ionophores, steroidal implants, and β-agonists, the Zn requirements of modern cattle may not be well understood. Understanding the impact of genetic background, breed, cattle age, size, and body composition on the Zn requirement and potential synergy with RAC will allow the beef industry to strategically supplement Zn in order to optimize productivity.

Zinc supplementation in this study was between 1 to 8 times the documented requirement (30 mg Zn/kg DM; NRC, 2000) and was provided from a source of Zn (Availa-Zn) that has been shown in broilers to be more bioavailable than ZnSO₄ (Star et al., 2012). The maximum tolerable Zn concentration is 500 mg Zn/kg DM (NRC, 2000), and research has noted no negative performance response to inorganic Zn supplementation up to 620 mg/kg DM (Beeson et al., 1977). Increasing dietary Zn using organic sources between 5 to 8 times the recommendations had little influence on growth performance or carcass characteristics. Although plasma Zn linearly increased as ZnAA supplementation

Table 6. The effect of supplemental Zn for 79 d and ractopamine hydrochloride supplementation (300 mg·steer⁻¹d⁻¹) for 31 d on finishing steer meat mineral, steak tenderness and meat collagen content from the 48 steers utilized for rib section collection in GRP2¹

Variable	Treatments ²						SEM	Contrast <i>P</i> -values ³		
	CON	ZnAA0	ZnAA60	ZnAA90	ZnAA120	ZnAA150		LZn	QZn	CON vs. Zn
Pens	4	4	4	4	4	4				
Meat mineral ⁴ , mg/kg DM										
Cu	3.9	2.3	2.9	3.0	3.4	3.1	0.34	0.07	0.47	0.02
Fe	59	59	59	66	74	55	3.8	0.43	0.08	0.42
Zn	135	143	136	149	161	142	7.0	0.37	0.77	0.14
Cooked steak WBSF ^{5,6} , kg	4.13	4.16	4.28	4.36	4.50	4.61	0.291	0.25	0.83	0.44
Meat collagen ⁷										
Total, mg/g wet tissue	3.75	5.00	3.42	3.67	3.98	4.36	0.307	0.18	0.002	0.33
Soluble, % of total ⁸	12.5	13.6	13.9	14.3	17.2	13.6	2.86	0.61	0.87	0.48

¹Two randomly selected animals were sampled per pen from GRP2.

²Treatments: a dry-rolled corn-based diet supplemented with 0 mg Zn/kg from ZnSO₄ and no supplemental ZnAA (CON; analyzed 30 mg Zn/kg DM), 60 mg Zn/kg from ZnSO₄ and no supplemental ZnAA (ZnAA0; analyzed 95 mg Zn/kg DM) or ZnAA0 diet supplemented with 60 (ZnAA60; analyzed 152 mg Zn/kg DM), 90 (ZnAA90; analyzed 188 mg Zn/kg DM), 120 (ZnAA120; analyzed 209 mg Zn/kg DM) or 150 (ZnAA150; analyzed 230 mg Zn/kg DM) mg Zn/kg DM from ZnAA.

³Contrasts: LZn = the linear effect of ZnAA; QZn = the quadratic effect of ZnAA. CON vs. Zn = CON vs. ZnAA0, ZnAA60, ZnAA90, ZnAA120, and ZnAA150.

⁴Rib sections were collected at abattoir after a 48 h chill.

⁵WBSF = Warner-Bratzler shear force.

⁶Steaks were vacuum packaged and aged in a 2°C cooler for 14 d, and then frozen until analysis.

⁷Meat sampled were collected 2 d postharvest, and immediately frozen until analysis.

⁸Data were natural log transformed for analysis, back-transformed data are presented.

increased, all plasma and liver Zn concentrations were within the adequate range (0.8 to 1.4 mg/L for plasma Zn, 25 to 200 mg/kg DM for liver Zn; Kincaid, 2000). Even as dietary Zn concentrations reached 230 mg/kg DM, the lack of changes in tissue Zn concentrations suggests that homeostatic mechanisms were not being overwhelmed, leading to rapid increases in Zn accumulation, as would occur with pharmacological concentrations of Zn (Spears and Hansen, 2008).

There is a well-known interaction between Cu and Zn, where increasing Zn absorption leads to an increase in the expression of the storage protein metallothionein, preventing absorption of excess Zn into the body and instead remaining in the gut epithelium to be sloughed off and excreted (Irató and Albergoni, 2005). However, metallothionein also has a high affinity for Cu, so when excess Zn is consumed this can also decrease the absorption of Cu (Hall et al., 1979). Plasma Cu and d 45 liver Cu concentrations were greater in CON steers than Zn-supplemented steers, although Cu concentrations for all treatments were within the adequate range (0.6 to 1.1 mg/L for plasma Cu) as described by Herdt and Hoff (2011) and the adequate (125 to 600 mg/

kg DM for liver Cu) to high range (0.9 to 1.1 mg/L for plasma Cu) as defined by Kincaid (2000). Day 45 liver Zn concentrations were lesser in CON steers than Zn supplemented steers, but again were well within the adequate range (25 to 200 mg/kg DM for liver Zn; Kincaid, 2000). Interestingly, CON had greater liver Cu and Zn concentrations based on samples collected at harvest. Liver biopsy sites and site of samples collected at the abattoir were different, and sampling location has been noted to have an impact on TM concentrations (Hickock et al., 1996), which may have led to these differences. Consultant recommended concentrations of Cu were supplemented in this study (Samuelson et al., 2016) but were consistent among treatments, leading to different dietary Zn:Cu ranging from 1.6 in the CON diet to 12.2 in the ZnAA150 diet. Overall, increasing dietary Zn concentration did decrease plasma and liver Cu but was not great enough to elicit a change in status, suggesting that perhaps the dietary Cu-to-Zn ratio is not critical in finishing steers with adequate Cu status.

Liver samples were also collected from all steers that were selected for rib section collection, allowing for a comparison between an indicator of TM status, and meat TM concentrations. Interestingly, there

was an increase in meat Cu concentration in CON that was similar to the trend observed for liver Cu concentrations. Altering meat TM concentrations using dietary supplementation has proven difficult. Ott et al. (1966) reported that increasing dietary Zn up to 2.1 g/kg DM elicited no change in muscle Zn concentrations. Yearling cattle supplemented with 360 mg Zn/day from various sources did not have different muscle Cu or Zn concentrations than unsupplemented cattle (Rojas et al., 1996). However, there was also a tendency for meat Cu to increase as ZnAA supplementation increased in the present study. Meat Cu concentration has been demonstrated to have a negative correlation with WBSF values, and a positive correlation with initial tenderness as rated by a trained sensory panel (Garmyn et al., 2011). Changes in Cu concentrations may lead to differences in meat tenderness. However, in this study, WBSF was not correlated with meat TM concentrations or collagen concentrations.

Zinc is an important component of matrix metalloproteinases (MMP), enzymes that break-down the extracellular matrix (Nagase et al., 2006). Degradation and synthesis of muscle through increased extracellular matrix turnover is necessary for growth, and require activity of MMP (Purslow et al., 2012), and increased MMP activity is found in lambs with high growth rates (Sylvestre et al., 2002). Alternatively, Zn may negatively impact activity of Ca-dependent proteases. Although a decrease in the activity of Ca-dependent proteases, the calpains, can be associated with increased growth rates in response to genetic mutations (Koochmaraie et al., 1995) and β -agonist administration (Koochmaraie et al., 1991), it may also lead to decreases in beef tenderness. Infusing lamb carcasses with ZnCl_2 increased 14 d WBSF and prevented the normal decrease in protease inhibitor activity during the aging process (Koochmaraie, 1991). Positive correlations between WBSF and muscle Zn concentrations have also been noted (Seideman et al., 1988); however, a direct relationship between dietary Zn concentration and muscle Zn concentration has not been established. In the current study, increasing total Zn concentrations up to 230 mg/kg DM had no effect on meat Zn concentrations or WBSF measurements. Similarly, Paulk et al. (2014) reported that increasing pig dietary Zn from 75 up to 225 mg/kg DM did not change WBSF of the loin, and supplementing 45 mg Zn/kg DM from various sources also did not influence muscle Zn concentration, meat quality or tenderness from bulls (Kessler et al., 2003). However, in the present study, there was a numerical trend for WBSF to increase as dietary Zn increased.

Despite rib sections only being collected from steers in GRP2, growth was very similar between steers that were sampled and all experimental steers, indicating they were representative of the entire experiment. Interestingly, all treatments had WBSF values that indicated steaks were tougher than the value to ensure a high level (98%) of consumer acceptability (4.1 kg; Huffman et al., 1996). The numerical increase in WBSF indicates that additional research should further define the relationship between dietary Zn concentration and WBSF.

Zinc supplementation did not affect growth performance prior to RAC supplementation in this study, as is consistent with previously completed work (Genther-Schroeder et al., 2016b). In both of these studies, steers were supplemented with Zn for 42 to 49 d prior to beginning RAC. Other work has also demonstrated that supplementing 1 g Zn from Zn-propionate per steer daily for 35 d prior to beginning RAC supplementation (400 $\text{mg}\cdot\text{steer}^{-1}\cdot\text{day}^{-1}$ for 28 d) led to no differences in growth performance in response to Zn supplementation (Edenburn et al., 2016). Alternatively, when ZnAA was supplemented at 0, 30, 60, or 90 mg Zn/kg DM for 86 d, steers receiving 60 mg Zn/kg DM had greater ADG than other treatments (Genther-Schroeder et al., 2016a). The differences in days of Zn supplementation prior to RAC supplementation may have contributed to the differences in performance. More work is needed to clarify the duration of increased Zn supplementation necessary relative to RAC feeding to see a performance response.

IMPLICATIONS

In contrast to previous work, no differences due to ZnAA supplementation were noted in the present study. When compared to previous work using similar dietary treatments, cattle in this study finished at a lighter BW, and had smaller REA, despite having similar BF, suggesting that cattle of differing composition may respond to ZnAA and RAC supplementation differently. Additionally, although dietary Zn supplementation up to 230 mg/kg DM did not influence Zn status, WBSF of cooked steaks was trending upwards, and may suggest that feeding higher concentrations of Zn, as is a trend in the industry, should be more carefully investigated with meat quality in mind. Overall, further research should be completed to evaluate how cattle type, genetics, composition, and mineral status impact mineral requirements and the response to growth technologies.

Conflict of interest statement. None declared.

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