The influence of supplemental zinc and ractopamine hydrochloride on trace mineral and nitrogen retention of beef steers¹

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ABSTRACT: The study objective was to determine whether N retention was improved with supplemental Zn above NRC concentrations with or without ractopamine hydrochloride inclusion. Angus cross-bred steers ($n = 32$, 485 ± 26 kg BW) with Genemax gain scores of 4 or 5 were utilized in a 2×2 factorial arrangement (8 steers/treatment). Steers were blocked by BW to a finishing diet with 1 of 2 mineral supplementation strategies (ZNTRT), no supplemental Zn (analyzed 32 mg Zn/kg DM; CON) or supra-nutritional Zn (CON + 60 ppm ZnSO_4 + 60 ppm Zn-amino acid complex; analyzed 145 mg Zn/kg DM; SUPZN), fed 56 days in pens equipped with GrowSafe bunks and assigned to β -agonist (BA) supplementation strategies of 0 (NON) or 300 mg steer⁻¹ d⁻¹ ractopamine hydrochloride (RAC) fed the last 30 d before harvest. Initial 56-d ADG was not affected by ZNTRT ($P = 0.66$), but DMI was greater in CON vs. SUPZN ($P < 0.01$). On day 56 (day 1 of BA supplementation), steers (4 groups; 8 steers/group; 2 steers/treatment) were moved to metabolism crates and adapted for 10 d, followed by 5 d of total fecal and urine collection. Total retention of Zn, Mn, Fe, Cu,

and N were calculated. Data were analyzed as a 2×2 factorial arrangement, with group as a fixed effect and the 3-way interaction of ZNTRT $\times$ BA $\times$ group as random. No interactions between ZNTRT and BA were noted for any data ($P \geq 0.19$). Collection DMI did not differ among treatments ($P \geq 0.23$); however, Zn intake was lesser in CON vs. SUPZN ($P < 0.01$). Fecal and urinary Zn excretion and Zn and Mn retention were lesser in CON vs. SUPZN ($P \leq 0.03$); however, Zn retention was not different between NON and RAC ($P = 0.43$). Retention of Cu and Fe was unaffected by strategies ($P \geq 0.49$). Urine output and urine N excretion were greater in NON vs. RAC ($P \leq 0.05$). Nitrogen retention (as percent of N intake) was lesser ($P = 0.05$) in CON (40.0%) vs. SUPZN (44.3%) and lesser ($P = 0.02$) in NON (39.5%) vs. RAC (44.8%). Zinc and N retention were found to be positively correlated ($r = 0.46$, $P < 0.01$). Average daily gain and G:F across the 86-d trial were lesser in NON vs. RAC ($P < 0.03$). Overall, SUPZN appears to improve N retention, suggesting that increasing dietary Zn may be important for cattle growth beyond that induced by ractopamine hydrochloride.

Key words: beef cattle, nitrogen, ractopamine hydrochloride, zinc

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INTRODUCTION

The trace mineral Zn is critical in numerous biological growth processes. Current recommendations of 30 mg Zn/kg DM were established more

than 50 yr ago to prevent deficiency in healthy animals and support growth (National Academies of Sciences, Engineering, and Medicine, 2016); however, beef cattle ADG from birth to slaughter has increased by 44% since 1977 (Capper, 2011). Genetics, improved management practices, and growth technologies have all contributed to this increase in cattle growth. Ractopamine hydrochloride (HCl), a β -adrenergic agonist, increases animal growth rates when fed 28 to 42 d prior

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to harvest (Ricks et al., 1984; Abney et al., 2007; Gruber et al., 2007) and is one reason for continuous improvement in cattle growth performance in the United States. In swine, increasing concentrations of lysine in the diet improves growth (Ross et al., 2011), and improvements in protein utilization regardless of CP content (Xiao et al., 1999) have been seen when ractopamine HCl is fed.

Nitrogen retention has been shown to increase in Holstein steers receiving supplemental ractopamine HCl (Walker et al., 2007), and Zn has long been established as critical in protein utilization in the body (Oberleas and Prasad, 1969; Somers and Underwood, 1969; Greeley et al., 1980). Genther-Schroeder et al. (2016a,b) reported that supplementation of Zn at 150 mg Zn/kg DM increased growth performance in steers receiving ractopamine HCl as compared to steers supplemented Zn at 90 mg Zn/kg DM. In these studies, additional growth responses to Zn were only noted in cattle receiving ractopamine HCl, suggesting an interaction between ractopamine HCl action and Zn may exist. However, it is unclear if this is an interaction in the β -adrenergic signaling cascade or if Zn requirements are increased due to greater growth rate induced by ractopamine HCl.

The objective of this study was to ascertain the impacts of ractopamine HCl and dietary Zn supplementation on N and Zn retention in beef steers. The hypothesis was that supranutritional supplementation of Zn would increase both Zn and N retention in steers consuming ractopamine HCl.

MATERIALS AND METHODS

All procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (8-15-8073-B).

Experimental Design

Cattle consisting of 75% or greater Angus genetic background from 2 separate sources were acquired and gentled for 1 mo prior to application of initial treatment. Gentling steers consisted of frequent handling, haltering with rope halters, and decreasing flight zone of the animal. The study was conducted as a 2×2 factorial, with mineral (ZNTRT) supplementation strategies of no supplemental Zn (analyzed 32 mg Zn/kg DM; CON) or supranutritional Zn [CON + 60 ppm ZnSO_4 + 60 ppm Zn-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN), analyzed 145 mg Zn/kg DM; SUPZN] beginning on day 0 and BA strategies of 0 (NON) or 300 mg steer⁻¹ d⁻¹ ractopamine HCl

(RAC; Actogain45, Zoetis, Parsippany, NJ) beginning on day 56. Steers ($n = 32$; 485 ± 26 kg) with Genemax gain scores of 4 or 5 (Zoetis), indicating the top 40% in growth potential of Angus cattle, were utilized in this study. Steers were separated into 4 groups ($n = 8$; 2 per treatment combination) and stagger-started on diets to accommodate space limitations in the metabolism facility. On day 0 for each group, steers were blocked by BW and Genemax scores to receive ZNTRT diets for 56 d in pens equipped with GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). Diet composition and analysis is shown in Table 1. On day 0 of the study, steers were implanted with Component TE-IS with Tylan (80 mg trenbolone acetate, 16 mg estradiol USP, and 29 mg tylosin tartrate; Elanco Animal Health, Greenfield, IN). Steers were weighed prior to feeding on day -1 and 0 and day 55 and 56 to determine initial weights and final weights of the feedlot period, respectively. After weighing on day 56, steers were transported 6.3 km to the metabolism facility in Kildee Hall (Iowa State University, Ames, IA). Steers continued to receive their respective ZNTRT diets in the metabolism facility from day 56 to 71 (day 0 to 15 of metabolism period). Within ZNTRT strategy steers were randomly assigned to BA strategies fed from day 56 to 84.

Table 1. Diet ingredient composition and nutrient content

Ingredient	CON diet (% DM)
Cracked corn	62
Modified distillers grains with solubles	25
Hay	8
Micronutrients and carrier ¹	5
Analyzed components	
Crude protein ² , %	14.6
NDF ² , %	19.2
Ether extract ² , %	5.19
Cu, mg/kg DM	12
Fe, mg/kg DM	164
Mn, mg/kg DM	33
Zn ³ , mg/kg DM	32

¹Basal includes dried distillers grains with solubles as carrier and micronutrients provided as % DM; limestone (1.5%), rumensin (0.0135%), and salt (0.31%). Trace minerals and vitamins provided per kilogram of DM: 0.15 mg Co (cobalt carbonate), 10 mg Cu (copper sulfate), 20 mg Mn (manganese sulfate), 0.1 mg Se (sodium selenite), 0.5 mg I (calcium iodate), and vitamin A 2,200 IU [ROVIMIX A 1000 (1,000 kIU/g), DSM, Parsippany, NJ].

²Chemical analysis completed by Dairyland Laboratories (Arcadia, WI).

³Control (CON) diet received no supplemental Zn (32 mg Zn/kg DM); supranutritional Zn (SUPZN) diet received formulated Zn inclusion of 120 mg Zn/kg DM [CON + 60 ppm ZnSO_4 and 60 ppm Availa-Zn (Zinpro Corporation, Eden Prairie, MN), which contains (DM basis) 35.5% Zn and AA complex]. CON diet analyzed 32 mg Zn/kg DM; SUPZN diet analyzed 145 mg Zn/kg DM.

Metabolism Period

From day 56 to 71 (day 1 to 10 adaptation, day 11 to 15 collection), steers (570 ± 30.9 kg BW) were housed in individual [213.4 cm (length) $\times$ 182.9 cm (height) $\times$ 91.4 cm (width)], stainless steel crates with rubber fatigue mats. Each morning, steers were offered the appropriate BA supplement (either 0.226-kg dried distiller grains without ractopamine HCl or 0.226-kg premix of dried distillers grains with ractopamine HCl included at 300 mg steer⁻¹ d⁻¹, respectively) along with 1.362 kg of the appropriate ZNTRT total mixed ration (TMR). The initial offering of TMR and BA supplement was consumed by steers before remaining daily TMR was offered. Feed delivery was 110% of the previous day's intake. All offered feed and refused feed for each steer were recorded daily, and daily intake was determined by subtracting refused feed from the offered feed. During the acclimation period, cattle adjusted to crates and allowed space to lie down. On the morning of day 10 (day 66 of study) of metabolism period, cattle were removed from crates, and crates were thoroughly cleaned. Preparation of metabolism crates prior to return of the steers, as well as daily fecal and urine collection procedures, was as described by [Pogge et al. \(2014a\)](#).

During the collection period (day 11 to 15; day 67 to 71 of study), feed orts were removed and weighed, and aliquots (≥ 600 g, as-fed) were collected. Control and SUPZN TMR as well as NON and RAC premixes were sampled daily. All feed and orts samples were dried in a convection oven at 70 °C for 48 h. Fecal and urine aliquots were collected as described by [Pogge et al. \(2014a\)](#) with the modification of a 3% aliquot of daily urine output. Determination of fecal DM was achieved according to procedures described by [Pogge et al. \(2014a\)](#). Dried fecal, TMR, and orts samples were subsequently ground through a 2-mm screen (Wiley Mill; Thomas Scientific, Swedesboro, NJ; Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) and stored in airtight bags until analysis.

On day 15 (day 71 of study) of the metabolism period, steers were removed from metabolism crates and transported 6.3 km to the Iowa State Beef Nutrition Farm, where they continued to receive their respective diets from day 71 to 86 of study. Prior to fecal collection and subsampling on day 71, crates were hand-scraped with acid-washed plastic paint scrapers and deionized water to collect all remaining feces excreted during the collection period.

Prefeeding weights were collected on day 85 and 86 to determine final BW. A 4% pencil shrink was applied to all live weights recorded during the trial. Steers were harvested at a commercial abattoir (Iowa Premium Beef, Tama, IA), and individual animal ID was maintained throughout slaughter. Liver samples were collected at harvest for analysis of mineral content.

Analytical Procedures

Total mixed ration samples of each diet were collected weekly during the feedlot period and after steers returned to the feedlot. Samples were dried for 48 h at 70 °C, and the resulting DM value was multiplied by as-fed feed intake to determine individual steer DMI during the feedlot period. Dry matter and OM of feed, orts, and fecal matter were determined according to [Association of Official Analytical Chemists \(AOAC, 1990\)](#) procedures. Nitrogen content of feed, orts, fecal matter, and urine was determined using the combustion method (TruMac N, LECO Corporation, Saint Joseph, MI; [Lundy et al., 2015](#)). Nutrient digestibility (DM, OM) was calculated as described by [Pogge et al. \(2014a\)](#). Nitrogen digestibility was calculated as described by [Lundy et al. \(2015\)](#).

Dried, ground, and composited feed, orts, and fecal samples were acid digested prior to mineral analysis according to the methods described by [Pogge et al. \(2014a\)](#). Liver samples were digested according to [Pogge et al. \(2014a\)](#). Urine samples were diluted 1:2 with 2% trace metal grade nitric acid for the analysis of Cu, Fe, Mn, and Zn. No additional dilutions were necessary for mineral analysis of feed, orts, or fecal matter for Cu, Fe, Mn, and Zn. Mineral analysis was conducted using inductively coupled plasma optical emission spectrometry (Optima 7000 DV, Perkin Elmer, Waltham, MA). A bovine liver standard from the National Institute of Standards and Technology (Gaithersburg, MA) was utilized to verify instrument accuracy, and yttrium (Inorganic Ventures, Christiansburg, VA) was used as an internal standard to account for any variation in sample introduction within a run.

Analyzed trace mineral concentrations (mg/kg or mg/L) were multiplied by total amounts of feed, orts, fecal matter, and urine, to determine total mineral content of each. Mineral intake was calculated by subtracting mineral refused from mineral consumed during the collection period. Daily mineral intake, fecal mineral output, and urine mineral output were determined by dividing total mineral content of each

by number of days of collection. Apparent absorption was determined by subtracting fecal mineral content from mineral intake, dividing by mineral intake, and multiplying by 100. Mineral retention was determined by subtracting excreted mineral (urine and fecal mineral) from mineral intake. Mineral retention as a percentage of intake was determined by subtracting retained mineral from consumed mineral, divided by mineral intake, and multiplying by 100.

Statistical Analysis

All data were analyzed as a randomized complete design. Growth and intake data for the initial 56-d period prior to entering metabolism crates (steer as experimental unit; $n = 16$ per ZNTRT) were analyzed using the Mixed procedures of SAS (SAS Institute Inc., Cary, NC). The model included the fixed effect of ZNTRT and group. Dry matter intake data were analyzed as repeated measures with week as the repeated effect. Data collected from day 56 onward were analyzed as a 2×2 factorial arrangement utilizing the Mixed procedure of SAS. Pearson correlation analyses (PROC CORR) was used to identify and quantify the relationship between Zn retention and N retention. The model for the analysis of the metabolism period, final BW, and liver mineral included the fixed effects of ZNTRT, BA, group, and the interaction of ZNTRT $\times$ BA, with the 3-way interaction of ZNTRT $\times$ BA $\times$ group as random. Steer was the experimental unit ($n = 8$ per treatment combination) for all analyses. Data for a steer from SUPZN–NON were removed from collection analysis due to negative retention values for Cu, Fe, Mn, and Zn throughout the collection period. Significance was declared at $P \leq 0.05$ and tendencies identified at $P = 0.06$ to 0.10 . Values reported are least square means and SEM.

RESULTS

Pre-BA Growth Period

After 56 d of Zn supplementation, there was no treatment $\times$ time interaction ($P = 0.99$) for DMI. Dry matter intake was increased ($P \leq 0.01$; Table 2) in CON vs. SUPZN. No differences in BW, ADG, or G:F were detected for the initial 56-d period ($P \geq 0.22$).

Collection Period

There were no interactions detected between ZNTRT $\times$ BA during the collection period ($P \geq 0.19$) for any variable. No effects of ZNTRT or BA were detected for DMI, DM digestibility, OM digestibility, N intake, fecal output and fecal N, or mg

Table 2. Dietary Zn influence on 56-d performance preceding metabolism period

Item	ZNTRT ¹		<i>P</i> value	SEM
	CON ²	SUPZN ³		
Steers (<i>n</i>)	16	16		
DMI ⁴ , kg/d	12.8	12.1	0.01	0.36
BW ⁵ , kg				
Day 0	484	487	0.68	4.0
Day 56	570	570	0.99	5.6
ADG ⁵ , kg				
Day 0 to 56	1.53	1.49	0.66	0.064
Gain to feed ⁵	0.114	0.120	0.37	0.0124

¹ZNTRT (mineral supplementation strategy).

²CON (no supplemental Zn; analyzed 32 mg Zn/kg DM).

³SUPZN [CON + 60 ppm ZnSO₄ + 60 ppm zinc-amino acid complex (Avala-Zn; Zinpro, Eden Prairie, MN), analyzed 145 mg Zn/kg DM].

⁴Daily DMI, repeated measures (no treatment $\times$ time interaction).

⁵All BW values include 4% pencil shrink in calculations.

N retained/d ($P \geq 0.20$; Table 3). Urine output was increased ($P = 0.05$) and urine N output was increased ($P = 0.01$) for NON vs. RAC. Nitrogen retention as a percentage of N intake was lesser in NON (39.7%) vs. RAC (44.8%; $P = 0.02$; Table 3). In addition, less N was retained as a percentage of N intake for CON (40.0%) vs. SUPZN (44.4%; $P = 0.05$).

Influence of ZNTRT and BA strategies on mineral intake, excretion, apparent absorption, and retention values on a milligram per day and percentage of intake basis is reported in Tables 4 and 5, respectively. Dietary concentrations of Zn did not influence intake, excretion, absorption, or retention of Cu or Fe when measured as milligram per day ($P \geq 0.24$) or as a percentage of intake ($P \geq 0.25$). Intake, fecal excretion, urinary excretion, and retention of Zn (mg/d) were lesser in CON vs. SUPZN ($P \leq 0.01$). Urinary Zn excretion (%) was greater in CON vs. SUPZN ($P \leq 0.01$). A positive correlation between Zn retention and N retention was detected ($r = 0.46$, $P < 0.01$). There was a tendency for increased fecal excretion of Mn ($P = 0.06$) and lesser apparent absorption ($P = 0.06$) and retention ($P = 0.05$) of Mn as a percentage of intake in CON vs. SUPZN. Intake of Mn (mg/d) tended to be lesser ($P = 0.06$) in CON vs. SUPZN, and Mn retention (mg/d) was decreased ($P = 0.03$) in CON vs. SUPZN. The BA strategy had no effect on trace mineral fecal excretion, urine excretion, apparent absorption, or retention when reported as milligram per day ($P \geq 0.29$) or as a percentage of intake ($P \geq 0.25$).

Overall Performance and Liver Mineral Concentrations

No ZNTRT $\times$ BA interactions were detected for overall performance data ($P \geq 0.17$). Across the feeding

Table 3. Influence of dietary Zn and ractopamine supplementation on DMI, diet digestibility, and daily urine and fecal output during 5-d collection period

Dietary treatment	ZNTRT ¹		<i>P</i> value ⁴	BA ¹		<i>P</i> value ⁴	SEM
	CON ²	SUPZN ²		NON ³	RAC ³		
Steers (<i>n</i>)	16	15		15	16		
DMI ⁵ , kg/d	9.43	10.12	0.40	9.89	9.67	0.78	0.558
DMD ⁶ , %	80.71	80.76	0.97	81.09	80.38	0.61	0.946
OMD ⁷ , %	81.95	82.19	0.84	82.49	81.65	0.51	0.869
N intake, g/d	210.3	224.0	0.45	218.3	216.0	0.90	12.31
Daily output							
Fecal, kg DM/d	1.85	1.96	0.66	1.90	1.91	0.98	0.173
Urine, L/d	8.85	8.56	0.82	10.13	7.23	0.05	0.876
Fecal, N g/d	46.8	49.8	0.58	48.2	48.4	0.97	3.79
Urine, N g/d	78.5	75.8	0.58	84.5	69.8	0.01	3.35
N retention, g/d	85.1	98.8	0.20	86.1	97.8	0.27	7.03
N retention ⁸ , %	40.0	44.3	0.05	39.5	44.8	0.02	1.38

¹ZNTRT (mineral supplementation strategy); BA (β-adrenergic agonist supplementation strategy).

²CON (no supplemental Zn; analyzed 32 mg Zn/kg DM); SUPZN [CON + 60 ppm ZnSO₄ + 60 ppm zinc-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN), analyzed 145 mg Zn/kg DM].

³NON (no supplemental ractopamine HCl); RAC (300 mg steer⁻¹ d⁻¹ ractopamine HCl; Actogain 45, Zoetis, Parsippany, NJ).

⁴ZNTRT × BA interaction was not significant (*P* ≥ 0.19).

⁵DMI over 5-d period during collection.

⁶DMD = DM digestibility.

⁷OMD = OM digestibility.

⁸Reported as percentage of intake.

Table 4. Influence of dietary Zn and ractopamine inclusion on daily micro mineral intake, fecal and urine excretion, and mineral retention of steers during 5-d collection period

Dietary treatment	ZNTRT ¹		<i>P</i> value ⁴	BA ¹		<i>P</i> value ⁴	SEM
	CON ²	SUPZN ²		NON ³	RAC ³		
Steers (<i>n</i>)	16	15		15	16		
Mineral intake							
Cu, mg/d	124	134	0.45	129	128	0.92	9.0
Fe, mg/d	1,617	1,678	0.71	1,656	1,640	0.92	111.4
Mn, mg/d	299	361	0.06	330	330	0.98	20.5
Zn, mg/d	322	1,534	<0.01	916	940	0.75	50.5
Fecal excretion							
Cu, mg/d	94	103	0.24	97	100	0.72	4.7
Fe, mg/d	999	1,004	0.96	1,005	998	0.94	66.1
Mn, mg/d	229	247	0.34	236	239	0.87	12.8
Zn, mg/d	206	1,004	<0.01	578	632	0.38	41.1
Urinary excretion							
Cu, mg/d	0.15	0.13	0.24	0.15	0.14	0.66	0.013
Fe, mg/d	1.92	1.98	0.77	2.00	1.90	0.65	0.160
Mn, mg/d	0.50	0.46	0.81	0.55	0.41	0.42	0.115
Zn, mg/d	1.09	1.99	<0.01	1.64	1.44	0.29	0.121
Mineral retention							
Cu, mg/d	29	31	0.87	32	28	0.71	6.7
Fe, mg/d	616	673	0.49	649	640	0.91	56.2
Mn, mg/d	70	114	0.03	93	90	0.86	12.1
Zn, mg/d	114	529	<0.01	337	306	0.43	26.8

¹ZNTRT (mineral supplementation strategy); BA (β-adrenergic agonist supplementation strategy).

²CON (no supplemental Zn, analyzed 32 mg Zn/kg DM); SUPZN [CON + 60 ppm ZnSO₄ + 60 ppm zinc-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN), analyzed 145 mg Zn/kg DM].

³NON (no supplemental ractopamine HCl); RAC (300 mg steer⁻¹ d⁻¹ ractopamine HCl; Actogain 45, Zoetis, Parsippany, NJ).

⁴ZNTRT × BA interaction was not significant (*P* ≥ 0.19).

Table 5. Influence of dietary Zn and ractopamine inclusion on daily micro mineral fecal and urine excretion, and mineral retention of steers as a percent of intake during 5-d collection period

Item	ZNTRT ¹		<i>P</i> value ⁴	BA ¹		<i>P</i> value ⁴	SEM
	CON ²	SUPZN ²		NON ³	RAC ³		
Steers (<i>n</i>)	16	15		15	16		
Fecal excretion							
Cu, %	78.2	78.9	0.89	76.7	80.5	0.49	3.67
Fe, %	62.0	60.8	0.61	60.6	62.2	0.53	1.71
Mn, %	76.7	69.4	0.06	71.9	74.2	0.50	2.32
Zn, %	64.9	65.7	0.83	63.1	67.4	0.25	2.45
Urinary excretion							
Cu, %	0.14	0.11	0.25	0.13	0.12	0.81	0.017
Fe, %	0.12	0.12	0.89	0.12	0.12	0.81	0.018
Mn, %	0.16	0.12	0.37	0.15	0.13	0.50	0.027
Zn, %	0.35	0.13	<0.01	0.26	0.22	0.41	0.035
Apparent absorption							
Cu, %	21.8	21.1	0.89	23.3	19.5	0.49	3.67
Fe, %	38.0	39.3	0.51	39.4	37.8	0.53	1.71
Mn, %	23.3	30.6	0.06	28.1	25.8	0.50	2.32
Zn, %	35.1	34.3	0.83	36.9	32.6	0.25	2.45
Mineral retention							
Cu, %	21.6	21.0	0.90	23.2	19.4	0.49	3.68
Fe, %	37.9	39.1	0.61	39.3	37.7	0.53	1.72
Mn, %	23.2	30.5	0.05	28.0	25.7	0.50	2.32
Zn, %	34.8	34.2	0.88	36.6	32.3	0.25	2.46

¹ZNTRT (mineral supplementation strategy); BA (β-adrenergic agonist supplementation strategy).

²CON (no supplemental Zn; analyzed 32 mg Zn/kg DM); SUPZN [CON + 60 ppm ZnSO₄ + 60 ppm zinc-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN), analyzed 145 mg Zn/kg DM].

³NON (no supplemental ractopamine HCl); RAC (300 mg steer⁻¹ d⁻¹ ractopamine HCl; Actogain 45, Zoetis, Parsippany, NJ).

⁴ZNTRT × BA interaction was not significant (*P* ≥ 0.19).

period, G:F and ADG were unaffected by ZNTRT strategy (*P* ≥ 0.30; Table 6). Both G:F and ADG were lesser in NON vs. RAC (*P* = 0.03). Liver mineral concentrations (mg/kg DM) were unaffected by both ZNTRT (*P* ≥ 0.15) and BA (*P* ≥ 0.30) strategies (Cu 296 ± 23, Fe 187 ± 17, Mn 8.0 ± 0.3, Zn 148 ± 13).

DISCUSSION

Previous research by Genther-Schroeder et al. (2016a,b) reveals increasing dietary Zn concentrations (as Zn-AA complex) of ractopamine HCl-fed cattle may result in improved growth. β-Adrenergic receptors, when activated, stimulate the membrane-bound enzyme adenylate cyclase, resulting in the production of cyclic adenosine monophosphate (cAMP; Lefkowitz et al., 1983). Cyclic AMP is a potent downstream intracellular messenger involved in the activation of cAMP-dependent protein kinase A (Yang and McElligott, 1989), eventually leading to the activation of hormone-sensitive lipase. As a result, BA act as repartitioning compounds, shifting anabolism in the late-stage finishing animal from adipose to protein accretion,

resulting in increased ADG, G:F, and HCW in pigs and cattle (Mersmann, 1998; Beermann, 2002; Johnson et al., 2014).

In swine, BA increase efficiency of dietary protein utilization (Xiao et al., 1999). This has been further refined in pigs, where increasing dietary lysine in conjunction with increasing supplemental ractopamine improves ADG and G:F (Webster et al., 2007; Ross et al., 2011). It is possible that feeding BA to livestock alters nutrient requirements; unfortunately, this is poorly understood in ruminants.

In the present study, N retention as a percentage of intake was independently improved by both ractopamine HCl supplementation and Zn supplementation. Ractopamine HCl supports cattle growth in part through increased N retention (Walker et al., 2007), likely due to a combination of decreased protein degradation (Hill and Malamud, 1974; Li and Jefferson, 1977; Tischler, 1981) and increasing protein accretion (Maltin et al., 1990). Wheeler and Koohmaraie (1992) theorized that increased muscle hypertrophy due to BA supplementation results from increased calpastatin activity, therefore decreasing proteolytic capacity. However,

Table 6. Influence of dietary Zn and ractopamine inclusion on performance measurements for day 0 to 86

Item	ZNTRT ¹		<i>P</i> value ⁴	BA ¹		<i>P</i> value ⁴	SEM
	CON ²	SUPZN ²		NON ³	RAC ³		
Steers (<i>n</i>)	16	16		16	16		
Final BW ⁵ , kg	600	604	0.54	598	606	0.23	4.38
Gain to feed ⁵	0.121	0.126	0.30	0.114	0.133	<0.01	0.0039
ADG ⁵ , kg	1.34	1.36	0.77	1.27	1.43	0.03	0.042

¹ZNTRT (mineral supplementation strategy); BA (β -adrenergic agonist supplementation strategy).

²CON (no supplemental Zn, analyzed 32 mg Zn/kg DM); SUPZN [CON + 60 ppm ZnSO₄ + 60 ppm zinc-amino acid complex (Availa-Zn; Zinpro, Eden Prairie, MN), analyzed 145 mg Zn/kg DM].

³NON (no supplemental ractopamine HCl); RAC (300 mg steer⁻¹ d⁻¹ ractopamine HCl; Actogain 45, Zoetis, Parsippany, NJ).

⁴ZNTRT $\times$ BA interaction was not significant (*P* $\geq$ 0.19).

⁵All BW values include 4% pencil shrink in calculations.

this phenethanolamine (L644,969) tested by these authors targets the β_2 -adrenergic receptor, whereas ractopamine HCl primarily targets the β_1 -adrenergic receptor (Anderson et al., 2005). In the present study, urine output was lesser in steers receiving ractopamine HCl, contributing to the lesser overall N excretion by ractopamine HCl-fed steers. It is unclear if others have noted similar effects of ractopamine HCl supplementation, as no studies completed with cattle supplemented ractopamine HCl report total urine output (Abney et al., 2007; Walker et al., 2007; Koontz et al., 2010). However, research in swine suggests a decrease in urine output can occur with ractopamine HCl supplementation (Ross et al., 2011).

Results of the present study not only support a role for ractopamine HCl in N retention but also suggest a critical role for Zn in N metabolism. Given the increased protein accretion experienced by β -adrenergic agonist-fed cattle, the positive correlation between N and Zn retention observed in this study suggests adequate Zn nutrition may be important in this growth response. Indeed, work in other species has displayed an interdependency of Zn and protein on growth, establishing that even with sufficient dietary protein, Zn is necessary for adequate protein utilization (Oberleas and Prasad, 1969; Greeley et al., 1980). Although the CON diet used in the present study met current supplementation recommendations for Zn (National Academies of Sciences, Engineering, and Medicine, 2016), increased N retention due to SUPZN suggests that the Zn requirement of feedlot cattle needs further refinement.

These results clearly define a role for Zn in bodily N retention, which is required for accretion and deposition of lean body mass. Increasing rate of growth due to growth-promoting technologies, genetic selection, and improved animal husbandry of modern cattle presents the opportunity for establishing supplemental Zn concentrations to optimize gain. However, increasing supplementation of Zn should

be approached with caution, as some countries have begun to place limits on supplementation rates based on concerns of excess manure concentrations (Jondreville et al., 2003). Determining the precise mechanisms by which Zn influences cattle growth is necessary to move the industry toward more strategic supplementation of Zn, potentially per unit of gain.

Increasing concentrations of Zn-AA increases plasma cAMP in cattle (Genther-Schroeder et al., 2016a), and increasing Zn-AA in combination with ractopamine HCl linearly increases BW, ADG, and G:F, suggesting an enhancement of the biological action of ractopamine HCl. Zinc has been shown to inhibit cyclic nucleotide phosphodiesterase (Spurlock et al., 1994), which is responsible for degrading cAMP and decreasing the response to BA (von Bülow et al., 2005; Haase and Rink, 2007). Zinc deficiency results in an increase in phosphodiesterase expression and a decrease in cAMP concentrations in mice (Hojyo et al., 2011). It has been suggested that Zn may interact with the β_2 -adrenergic receptor, potentiating cell signaling, and increasing internalization of the receptor; however, Hergenreder et al. (2016) reported that Zn fed as Zn methionine has no effect on internalization of the β_1 -adrenergic receptor, which ractopamine HCl preferentially binds to. As there was no interaction between BA and ZNTRT in the present study for measures of N retention, it appears there may be opportunity to utilize Zn to improve N retention in cattle even when ractopamine HCl is not fed.

Ractopamine HCl did not change the coefficient of absorption of Zn in the present study and does not appear to upregulate Zn absorption or retention. Coefficients of absorption for Zn in this study (34.5%) are greater than those reported in previous studies in beef steers [22.2% for receiving calves, Nockels et al., 1993; 10.0% (ZnSO₄) and 19.6% (ZnOHCl) for growing steers, Shaeffer et al., 2017; 16.0% for growing steers, Pogge et al., 2014a; and

9.9% for growing steers, Pogge et al., 2014b]. The reasons for the high coefficient of absorption of Zn in the present study are unclear. To the authors' knowledge, Zn absorption resulting from differing concentrations of Zn supplementation in late-stage finishing steers (570 ± 30.9 kg) has not been studied previously and may be reflective of stage of production or BW [167 ± 5 kg, Nockels et al., 1993; 371 kg, Shaeffer et al., 2017; 368 ± 12 kg (fistulated) and 388 ± 10 kg (unmodified), Pogge et al., 2014a; 370 ± 9.5 kg, Pogge et al., 2014b]. Growth in this period of production has been shown to shift away from protein accretion toward adipose tissue deposition. Previous studies have shown increased carcass attributes associated with adipose tissue with Zn supplementation (quality grade, marbling score, yield grade, and backfat; Greene et al., 1988; Spears and Kegley, 2002) and could possibly be influencing the effect on Zn absorption seen in the present study.

The lack of difference reported for the coefficient of absorption of Zn in SUPZN vs. CON is in contrast to those published previously in rats and chickens (Weigand and Kirchgessner, 1979; Mohanna and Nys, 1999), where increasing supplemental Zn concentrations decreased the coefficient of absorption. In general, Zn absorption is downregulated when dietary Zn is fed well above animal requirements, which may suggest that late-stage finishing steers require greater dietary Zn concentrations than currently recommended. In addition, steers in the SUPZN treatment retained a greater amount of Zn (mg/d) than CON steers, similar to previous reports in rats (Weigand and Kirchgessner, 1979), supporting that increasing supplemental concentrations of Zn increases the amount of Zn retained. Given the positive correlation observed in this study and others between Zn and N retention (Oberleas and Prasad, 1969; Greeley et al., 1980), it appears that increasing dietary Zn may increase N capture in animals and may be of particular value in rapidly growing individuals. Further work is needed to refine beef cattle requirements for Zn based on stage of production, BW, and rate of growth.

Supplementing ractopamine HCl at $300 \text{ mg} \cdot \text{steer}^{-1} \cdot \text{d}^{-1}$ did not change absorption or retention of Cu, Fe, or Mn. In addition, liver mineral concentrations of Cu, Fe, Mn, and Zn were unaffected by RAC or SUPZN. A well-established antagonism between dietary Zn and Cu exists, where Zn tends to increase tissue metallothionein expression, which then binds to Cu, decreasing Cu absorption (Oestreicher and Cousins, 1985). Regardless, no effects on Cu absorption due to SUPZN were noted in the present study. Metallothionein expression tends

to increase when transitioning to a greater concentration of supplemental dietary Zn (Cousins, 1985), but absorption and fecal excretion of endogenous Zn have been shown to adjust within 6 d of increasing supplemental concentrations in rats (Weigand and Kirchgessner, 1978). Therefore, adjustment to increased dietary concentrations of Zn may have preceded the metabolism period of the present study. Furthermore, ruminants store Cu very effectively in the liver (Suttle, 2010), and the cattle in this study had highly adequate Cu status according to harvest liver Cu concentrations; therefore, SUPZN may have had little relevant influence on Cu status. It is necessary to be cognizant of the relationship between Cu and Zn, as cattle requirements for both essential elements continue to be refined.

Interestingly, Mn absorption and retention were increased by SUPZN treatment. To the authors' knowledge, this has not been reported by others and may be due to late finishing stage of these animals, as few metabolism studies have examined beef steers of this weight. Manganese supports enzymes in N recycling (Hellerman and Perkins, 1935) and antioxidant capacity (McCord and Fridovich, 1969; Borgstahl et al., 1992). Absorption rates of Mn in the present study are greater than seen in previous work in humans (Greger et al., 1978) and cattle (Pogge et al., 2014b), and further work is needed to determine whether the findings of the present study are repeatable.

There is opportunity in the industry to refine feeding strategies for livestock when utilizing growth-promoting technologies. It does not appear that ractopamine HCl alters apparent absorption of trace minerals, suggesting that if increased retention of trace minerals such as Zn is needed for an optimal growth response to ractopamine HCl, greater dietary concentrations of trace minerals than those utilized in this study may be needed to provide these nutrients. Future work is needed to elucidate the mechanism behind increased N retention in beef steers due to SUPZN supplementation, but clearly Zn has a positive role in N retention. Further research is needed to move the industry toward more strategic supplementation of trace minerals to support optimum livestock production while concurrently lessening environmental impact.

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