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The Influence of Supplemental Zinc and Ractopamine Hydrochloride on the Performance and *Longissimus Thoracis* Proteome of Finishing Beef Steers

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Abstract: To determine how Zn and ractopamine hydrochloride (RAC) supplementation affect the *longissimus thoracis* muscle proteome in beef cattle, 48 high percentage Angus steers (494 ± 18.2 kg) were utilized in a 2 × 2 factorial study design. Steers were blocked by body weight (BW) and genetic gain potential (GeneMax; Zoetis, Parsippany, NJ) into control (CON; analyzed 36 mg Zn/kg dry matter [DM]) or supranutritional Zn (SUPZN; CON + 60 mg Zn/kg DM from $ZnSO_4$ + 60 mg Zn/kg DM from Zn-amino acid complex; Availa Zn, Zinpro, Eden Prairie, MN) dietary treatments (ZNTRT). Starting 28 d prior to harvest, steers were blocked by BW within ZNTRT to RAC treatments (RACTRT) of 0 (NO) or 300 mg·steer^{-1}·d^{-1} (RAC; Actogain 45, Zoetis). After 15 d on RACTRT, *longissimus thoracis* biopsies were collected from all steers for proteomic analysis. Twenty-eight steers ($n = 7$ per treatment) were harvested at a commercial abattoir on Day 90. A ZNTRT × RACTRT effect showed SUPZN-RAC steers had a greater dressing percentage than other treatments ($P \leq 0.02$). Steers fed SUPZN had a greater hot carcass weight, carcass-adjusted final BW, overall average daily gain, and overall gain to feed ($P \leq 0.05$). Differentially abundant proteins involved in energy metabolism, muscle structure, and protein synthesis potentially indicate muscle fiber characteristic differences because of Zn and RAC supplementation. Both myosin light chain kinase 2 and eukaryotic translation initiation factor 5A-1 were more abundant in SUPZN-RAC steers than SUPZN-NO ($P \leq 0.05$) or CON-NO ($P \leq 0.04$) steers when analyzed for phosphorylation modifications. L-lactate dehydrogenase B was more abundant in SUPZN-NO steers than CON-NO ($P = 0.03$) or CON-RAC ($P = 0.01$) steers. In conclusion, increased Zn supplementation may be needed to optimize the hypertrophic effects of RAC through its effects on the *longissimus thoracis* proteome.

Key words: beef cattle, beta adrenergic agonists, muscle, proteomics, zinc
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Introduction

Beef production in the United States has become increasingly efficient (USDA NASS, 2019) in part because of improvements in nutritional management strategies. These strategies include the use of β-adrenergic agonists, such as ractopamine hydrochloride (RAC). Approximately 85% of US feedlots utilize RAC as a growth-promoting technology (Samuelson et al., 2016). This β-adrenergic agonist has been shown to consistently improve average daily gain (ADG) and feed efficiency of cattle when supplemented 28 to 42 d before harvest (Bohrer et al., 2014). These effects are exerted through RAC activating β-adrenergic receptors on both skeletal muscle and adipose tissue, resulting in increased intracellular concentrations of cyclic AMP that bind to protein kinase A (Spurlock et al., 1994). This activation has downstream effects of decreasing protein degradation and increasing lipolysis (Wang and Beermann, 1988; Liu et al., 1989), causing lean muscle accretion associated with RAC supplementation.

Zn has also been shown to have intracellular effects required for bodily growth processes. Zn is needed intracellularly in the catalytic sites of RNA

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polymerases as well as in the Zn-finger protein domains for gene transcription (Dalton et al., 1997; Cousins, 1998). Additionally, Zn is able to increase protein synthesis in the muscle by phosphorylating and activating protein kinase B and mammalian target of rapamycin pathways (Kim et al., 2000; McClung et al., 2007). The supplementation of RAC with dietary Zn above the current recommendations (National Academies of Sciences, Engineering, and Medicine, 2016) has been shown to increase growth in cattle (Genther-Schroeder et al., 2016a, 2016b). Therefore, Zn may support RAC-enhanced growth processes, but the interaction of Zn and RAC occurring in the muscle is poorly understood and little is known about how RAC and supranutritional dietary Zn concentrations affect the muscle proteome of cattle, either independently or collectively. Therefore, the objective of this study was to determine how Zn and RAC supplementation affect the *longissimus thoracis* muscle proteome of beef steers. It was hypothesized that the abundance and phosphorylation state of proteins would be affected by supranutritional Zn and RAC supplementation to better support protein accretion, leading to increased muscle growth.

Materials and Methods

All procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (11-17-8645-B).

Animals and experimental design

Seventy high percentage Angus steers (approximately 12 mo old) from one source were purchased, transported to the Iowa State University Beef Nutrition Farm (Ames, IA), and tested for their genetic merit for growth, carcass weight, and quality grade (marbling) using GeneMax Gain scores (GeneMax Focus, Zoetis). After performing GeneMax test, 48 steers (494 ± 18.2 kg) were identified as being in the top 60% of Angus cattle for growth potential and were selected for enrollment in this study. Steers were housed (6 or 8 steers per pen) in pens equipped with GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) and fed ad libitum to allow for the assessment of daily individual steer feed disappearance.

Steers were divided into 5 groups of 4 (1 steer/treatment) and a sixth group of 28 (7 steers/treatment) to accommodate harvest schedules. On Day 0, steers were implanted with Component TE-S with Tylan (120 mg trenbolone acetate, 24 mg estradiol, United States Pharmacopeia, Rockville, MD, and 29 mg tylosin tartrate; Elanco Animal Health, Greenfield, IN). This study used a 2 × 2 factorial as its treatment design. Zn supplementation (ZNTRT) began on Day 0 and consisted of the recommended daily minimum requirement of Zn to prevent deficiency (analyzed 36 mg Zn/kg DM; CON) or supranutritional Zn [CON {36 mg Zn/kg DM} + 60 mg Zn/kg DM as $ZnSO_4$ + 60 mg Zn/kg DM as Zn-amino acid complex (Availa Zn, Zinpro), analyzed 140 mg Zn/kg DM; SUPZN]. Steers were blocked by similar GeneMax Gain scores and body weight (BW) on Day 0 before being randomly assigned to ZNTRT. Twenty-eight days prior to harvest (Day 62 of the trial), β-adrenergic agonists treatments (RACTRT) began, consisting of either 0 (NO) or 300 $mg^{-1}{\cdot}steer^{-1}{\cdot}d^{-1}$ ractopamine HCl (Actogain 45, Zoetis; RAC). Twenty-eight days prior to harvest (Day 62 on trial), steers within a ZNTRT assignment were randomly assigned to an RACTRT to be continued through the end of the trial. Diet composition and nutrient analysis is shown in Table 1. Diets were stagger-started over a 30-d period such that days on ZNTRT and RACTRT were identical across groups. Groups 1 through 5 (1 steer/treatment) were harvested at the Iowa State University Meats

Table 1. Diet ingredient composition and nutrient content

Ingredient (% DM)	CON[1]	SUPZN[1]
Dry rolled corn	57	57
Sweet bran[2]	20	20
Hay	8	8
Dried distillers grains with solubles	10	5
Zinc treatment premix[3]	0	5
Micronutrients[4]	5	5
Analyzed components		
Crude protein	13.1	13.4
Ether extract	3.8	3.8
Neutral detergent fiber	23.0	23.2
Zinc, mg/kg DM	36	140

[1]CON diet contained no supplemental Zn; SUPZN diet contained 60 mg supplemental Zn/kg DM as $ZnSO_4$ and 60 mg supplemental Zn/kg DM as Availa-Zn (Zinpro Corporation, Eden Prairie, MN).

[2]Branded wet corn gluten feed (Cargill Corn Milling, Blair, NE).

[3]Dried distillers grains plus solubles was used as carrier to deliver supplemental Zn treatment.

[4]To provide as a percent of diet DM: 3% dried distillers grains, 1.5% limestone, 0.31% salt, 0.0135% Rumensin 90. To provide per kg diet DM: 10 mg Cu/kg DM (copper sulfate), 20 mg Mn/kg DM (manganese sulfate), 0.1 mg Se/kg DM (sodium selenite), 0.15 mg Co/kg DM (cobalt carbonate), 0.5 mg I/kg DM (calcium iodate), 2,200 IU vitamin A and 25 IU vitamin E.

Laboratory (Ames, IA) on 5 separate days (approximately 17 mo old) and data from these groups, including postmortem data, are described in Schulte et al. (2021). The remaining 28 steers (7 steers/treatment), composing Group 6 (approximately17 mo old), were harvested on a single day at National Beef (Tama, IA).

Sample collection and analysis

Samples of the total mixed ration (TMR) for each diet were collected weekly, and dry matter (DM) content was determined by drying samples for 48 h at 70°C in a forced air oven. Resulting DM values were used to correct daily as fed feed disappearance for each steer to determine individual DM intake (DMI). Dried TMR samples were composited and sent to a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI) for nitrogen (CP; AOAC, 2005a; method 990.03), neutral detergent fiber (AOAC, 2005b; method 2002.04), and ether extract (AOAC, 2000; method 920.39) analysis.

Prior to feeding, steers were weighed on Day −1 and 0 (initial BW), Day 61 and 62 (prior to RACTRT), and Day 88 and 89 (final BW). Because of the difference in harvest conditions and chill time, only carcass data from Group 6 ($n = 28$; 7 steers/group) harvested at National Beef were included in this manuscript. Carcass data including hot carcass weight (HCW), back fat thickness (BF), area of the *longissimus thoracis* muscle (REA), marbling score (MS), and kidney, pelvic, and heart fat (KPH) were collected by trained university personnel after a 48-h chilling period. These data were used to calculate yield grade (YG) using the USDA YG equation [2.50 + (2.5 × adjusted fat thickness [in]) + (0.2 × kidney, heart and pelvic fat [%]) + (0.0038 × HCW [lb]) −(0.32 × ribeye area [in^2])]. Individual HCW was divided by the average dressing percentage (DP; 62.1%) to determine carcass-adjusted performance variables. A 4% calculated shrink was applied to all live BW measurements to account for gut fill, including those used to calculate ADG and gain-to-feed ratio (G:F).

Blood serum analysis

Blood samples were collected from each steer via jugular venipuncture prior to feeding on Day 62 (prior to RACTRT) and Day 89. Blood was collected in vacutainer tubes with sodium heparin, trace mineral grade K_2 ethylenediamine tetraacetic acid (EDTA), or no additives for serum (Becton Dickinson, Franklin Lakes, NJ). Serum tubes were allowed to coagulate at room temperature for 1 h prior to centrifugation, whereas heparin and trace mineral grade K_2 EDTA tubes were stored on ice to be transported to the laboratory. Heparin and trace mineral grade K_2 EDTA tubes were centrifuged at 1,000 × *g* for 20 min at 4°C, and serum tubes were centrifuged at 1,500 × *g* for 20 min at 4°C. The resulting plasma and serum were then aliquoted and stored at −20°C until further analysis.

Trace mineral analysis

Trace mineral analyses of TMR, plasma, and liver (collected at harvest) were conducted using inductively coupled plasma optical emission spectrometry (Optima 7000 DV, PerkinElmer, Waltham, MA). Prior to mineral analysis, TMR samples were acid digested according to (Pogge et al., 2014), whereas plasma and liver samples were digested and prepped for analysis according to Pogge and Hansen (2013). Instrument accuracy was verified by a bovine liver standard from the National Institute of Standards and Technology (Gaithersburg, MA) for TMR and liver analysis and a trace elements serum toxicology control (UTAK Laboratories, Valencia, CA) for plasma trace mineral analysis. Variation in sample introduction within runs was accounted for through the use of yttrium (PerkinElmer) as an internal standard.

Nonesterified fatty acid and plasma urea nitrogen analysis

Commercial colorimetric kits were used to determine serum nonesterified fatty acid content (NEFA; Wako Pure Chemical Industries Ltd., Chuo-ku, Osaka, Japan) and plasma urea nitrogen (PUN; urea nitrogen reagent, Teco Diagnostics, Anaheim, CA). Blood analysis inter-assay and intra-assay coefficient of variation values were 11.08% and 5.85% for NEFA data and 3.42% and 1.13% for PUN data, respectively.

Muscle biopsies

Longissimus thoracis muscle biopsies were collected between the 11th and 12th rib on Day 77 (Day 15 on RACTRT) from each steer using a method modified from Pampusch et al. (2008). Briefly, steers were restrained in a hydraulic squeeze chute, hair was removed at the incision cite and cleaned using 70% isopropanol. A betadine solution was used as a local anesthetic. A sterile scalpel was used to make an incision and a modified bone marrow biopsy probe was used to collect the sample (approximately 1 g). Samples were flash frozen in liquid nitrogen and stored at −80°C until further analysis. At the completion of

tissue collection, animals were returned to their original pen.

Sarcoplasmic protein extraction and protein identification

Frozen *longissimus thoracis* muscle from Day 77 biopsies were homogenized and powdered in liquid nitrogen, and sarcoplasmic proteins were extracted as described by Carlson et al. (2017) using sarcoplasmic extraction buffer (4°C: 50 mM Tris-HCl and 1 mM EDTA [pH 8.0]).

Sarcoplasmic extracts (200 µL; 10 mg/mL protein concentration) were submitted to the Iowa State University Protein Facility. A pooled control sample was prepared containing equal amounts of all samples. All samples were reduced with dithiothreitol, modified (Cys) with iodoacetamide, and digested with trypsin/Lys-C (Promega, Madison, WI) at 37°C overnight. The addition of formic acid stopped digestion before samples were dried in a Savant SpeedVac SC110A with a Thermo Refrigerated Vapor Trap RVT4104 (Thermo Scientific, Waltham, MA). Samples were desalted using C18 MicroSpin Columns (Nest Group, Southborough, MA) before drying again in a SpeedVac. Concentration of the samples was determined by the Pierce Colorimetric kit (Fisher Scientific, Hampton, NH) once they were reconstituted in 1 M triethylammonium bicarbonate. Each sample was labeled with TMT10plex reagents (Thermo Scientific), with the pooled control labeled with TMT11 (131C). Equal amounts of each sample from the individual TMT label and the control were pooled into a single tube and dried in a SpeedVac. Reconstitution of the samples was achieved by 5% acetonitrile/water/0.1% formic acid.

Peptides were separated by liquid chromatography (Thermo Scientific EASY nLC-1200 coupled to a Thermo Scientific Nanospray FlexIon source) through a pulled glass emitter 75 µm by 20 cm (Agilent, Santa Clara, CA). The tip of the emitter was packed with 5 µm SB-C18 packing material (Agilent), whereas the remainder of the column was packed with UChrom C18 3 µm material (nanoLCMS Solutions, Oroville, CA). A NanoSpray FlexIon source (Thermo Scientific) coupled to a Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer with a higher-energy C-trap dissociation fragmentation cell was used to perform MS/MS. The resulting intact mass and MS/MS fragmentation pattern was compared with MASCOT (Perkins et al., 1999) and Sequest HT (Tabb et al., 2001) theoretical fragmentation patterns in order to detect peptides that can identify proteins using Thermo Scientific's Proteome Discoverer 2.4 software. The MASCOT and Sequest HT search was run against Uniprot *Bos taurus*. These searches were performed with static modification of carbamidomethyl (Cys) and TMT label (Lys and N-termini), along with dynamic modifications of oxidation (Met) and deamidation (Asn, Gln). Another search was performed with the dynamic modification of phosphorylation (Ser, Thr). Data were sent to the Iowa State University Bioinformatics Facility for analysis as described as follows.

Statistical analysis of feedlot performance, carcass data, liver and blood parameters

Normality was confirmed using the Shapiro-Wilk test. For the pre-RAC period, performance data were analyzed as a randomized complete block design using the Mixed procedure of SAS (SAS Institute Inc., Cary, NC), with the experimental unit of steer ($n = 24$/ZNTRT) and fixed effects of group (1 through 6) and ZNTRT; Day 62 blood parameters were analyzed similarly using SAS. A 2×2 factorial arrangement was used to analyze RAC period live-animal performance as well as Day 89 blood parameters and liver trace mineral concentrations with the fixed effects of group (1 through 6), ZNTRT, RACTRT, and the interaction of ZNTRT and RACTRT ($n = 12$/treatment combination). Carcass data and carcass-adjusted performance were analyzed similarly ($n = 7$/treatment combination) but without the effect of group. Initial BW served as a covariate for HCW analysis. Cook's D was used to determine outliers at Cook's $D \geq 0.2$. Accordingly, 1 SUPZN-RAC, 3 SUPZN-RAC and 1 SUPZN-NO, and 1 SUPZN-RAC and SUPZN-NO were removed because they were identified as outliers from Day 62 plasma Zn, Cu, and Fe, respectively. Additionally, one SUPZN-RAC and CON-RAC were removed from Day 89 plasma Cu and Fe, respectively. Furthermore, one steer from CON-RAC was removed from all analyses because of health reasons unrelated to treatment. Significance was determined at $P \leq 0.05$ and tendencies declared at $0.06 \leq P \leq 0.10$. Tabular values are least square means, and pairwise differences were determined using the PDIFF statement in SAS.

Bioinformatics statistical analysis

For each peptide, the signal intensity was divided by the 131C intensity as the normalization factor before further analysis. Peptides with more than 50% missing value within each treatment group were not used. The remaining data were normalized using the interquantile

range estimation method. If there were 2 data points for that peptide from Sets 1 and 2, one data point was randomly selected. If there was only one data point for that peptide, that data point was accepted. This process was repeated for each peptide in each treatment group. MetaboAnalyst 4.0 (Chong et al., 2018) was used for data analysis.

Upon finding data integrity to be satisfactory (positive values for the area), missing value estimation was attributed using Singular Value Decomposition method (Pang et al., 2020). Noninformative values that were near-constant throughout the experiment conditions were detected using the interquantile range estimation method and deleted. Data were transformed based on generalized logarithm transformations to make individual features more comparable. Individual treatment comparisons were made by *t* test with the *P* value cutoff of 0.1. Log2 fold change was performed to compare treatments. The Uniprot protein IDs that were up/down regulated with the adjusted *P* values less than 0.1 were used to retrieve the corresponding KEGG IDs using the "Retrieve/ID mapping" tool of Uniprot. KEGG IDs were then used to retrieve biological pathway association of the proteins. Enrichment analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) 6.8 Tools (Huang et al., 2009).

Results

Day 0 to 62 feedlot performance, liver, and blood metabolites

Growth performance, liver, and blood metabolite results for the initial 62 d of the trial, prior to RACTRT, are reported in Table 2. There was no effect of ZNTRT on BW, ADG, DMI, or G:F ($P \geq 0.32$) during this time. As expected, Zn intake was greater in the SUPZN compared with the CON during this period ($P = 0.0001$), resulting in greater plasma Zn concentrations for SUPZN than CON ($P = 0.007$) on Day 62. However, plasma Cu and Fe concentrations were not affected by ZNTRT ($P \geq 0.32$). Furthermore, CON tended to have greater NEFA concentrations ($P = 0.06$) than SUPZN on Day 62, though ZNTRT did not affect Day 62 PUN concentrations ($P = 0.20$).

Day 62 to 89 feedlot performance, liver, and blood metabolites

Growth performance results for the final 28 d of the trial, during the RAC feeding period, are reported in Table 3. No ZNTRT, RACTRT, or ZNTRT × RACTRT effects were observed for BW or DMI ($P \geq 0.18$). A ZNTRT × RACTRT effect was observed for ADG and G:F ($P \leq 0.05$), in which CON-NO had lesser ADG and G:F than CON-RAC and CON-RAC ADG was greater than SUPZN-RAC. A tendency for a ZNTRT × RACTRT effect ($P = 0.08$) was observed in daily Zn intake during this period. Both CON-NO and CON-RAC had lesser Zn intake than SUPZN-NO and SUPZN-RAC, but SUPZN-NO had greater Zn intake than SUPZN-RAC.

Final liver trace mineral concentrations and blood metabolites during the RAC feeding period are reported in Table 4. No ZNTRT × RACTRT effects were observed in harvest liver or Day 89 plasma trace mineral concentrations ($P \geq 0.11$). Both harvest liver and Day 89 plasma Zn were greatest for SUPZN ($P \leq 0.005$), whereas RAC increased harvest liver Zn ($P = 0.04$) but not Day 89 plasma Zn concentrations ($P = 0.21$). Harvest liver Fe was not influenced by ZNTRT ($P = 0.12$) but Day 89 plasma Fe tended to be greater for SUPZN ($P = 0.07$). Furthermore, liver Fe was greater

Table 2. Effect of dietary Zn concentration on growth performance and blood analyses of steers for the 62 days prior to supplementation with ractopamine hydrochloride

	Treatment			*P*-value
Item	CON[1]	SUPZN[1]	SEM	ZNTRT
Steers (*n*)	23	24		
BW[2], kg				
d 0[3]	493	494	4.4	0.83
d 62[3]	647	651	4.2	0.45
ADG[2], kg	2.49	2.56	0.067	0.45
Dry matter intake, kg/d	13.8	13.7	0.42	0.94
Gain to feed	0.180	0.190	0.0080	0.32
Zn intake, mg/day	529	1880	57.1	0.0001
d 62 plasma trace mineral[4], mg/L				
Zn	1.19	1.32	0.038	0.007
Cu	0.88	0.88	0.028	0.99
Fe	2.11	2.21	0.086	0.32
NEFA[5], mEq/L	131.8	114.7	7.64	0.06
PUN[6], mg/dL	10.3	9.3	0.64	0.20

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg DM); SUPZN = CON + 60 mg Zn/kg DM from $ZnSO_4$ + 60 mg Zn/kg DM from Zn-AA complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN).

[2]4% shrink applied to all body weight calculations.

[3]Average of two consecutive day body weights.

[4]Plasma trace mineral on d 62; one steer removed from Zn ([1] SUPZN-RAC), four removed from Cu ([3] SUPZN-RAC and [1] SUPZN-No) and two removed from Fe ([1] SUPZN-RAC and [1] SUPZN-NO) analysis.

[5]Serum non-esterified fatty acids on day 62; one steer removed from CON due to lack of sample.

[6]Plasma urea nitrogen on day 62.

Table 3. Effect of dietary Zn and ractopamine hydrochloride on growth performance and Zn intake of steers during the ractopamine period

	CON[1]		SUPZN[1]			*P*-value		
Item	NO[2]	RAC[2]	NO[2]	RAC[2]	SEM	ZNTRT	RACTRT	ZNTRT x RACTRT
Steers (*n*)	12	11	12	12				
BW[3], kg								
d 62[4]	649	645	654	648	5.7	0.45	0.40	0.84
d 89[4]	707	720	721	713	8.1	0.68	0.72	0.18
ADG[3], kg	2.17[b]	2.77[a]	2.48[ab]	2.40[ab]	0.166	0.83	0.09	0.03
Dry matter intake, kg/d	14.9	15.6	15.4	15.5	0.59	0.68	0.47	0.54
Gain to feed	0.145[b]	0.178[a]	0.164[ab]	0.154[ab]	0.0118	0.80	0.30	0.05
Zn intake, mg/d	574[z]	617[z]	2203[x]	1999[y]	77.0	0.0001	0.24	0.08

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg DM); SUPZN = CON + 60 mg Zn/kg DM from $ZnSO_4$ + 60 mg Zn/kg DM from Zn-AA complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN).

[2]NO = no supplemental ractopamine hydrochloride; RAC = 300 mg·steer^{-1}·d^{-1} ractopamine hydrochloride (Actogain45; Zoetis, Parsippany, NJ) from day 62 to end of trial.

[3]4% shrink applied to all body weight calculations.

[4]Average of two consecutive day body weights.

[abc]Within a row, means with differing superscripts are different ($P \leq 0.05$).

[xyz]Within a row, means with differing superscripts tend to be different ($P \leq 0.10$).

Table 4. Effect of dietary Zn and ractopamine hydrochloride on liver and blood analyses of steers

	CON[1]		SUPZN[1]			*P*-value		
Item	NO[2]	RAC[2]	NO[2]	RAC[2]	SEM	ZNTRT	RACTRT	ZNTRT x RACTRT
Steers (*n*)	12	11	12	12				
Harvest liver, mg/kg DM								
Zn	127	134	140	161	7.3	0.005	0.04	0.28
Cu	285	312	254	257	25.3	0.07	0.53	0.59
Fe	140	166	152	184	10.4	0.12	0.003	0.74
Mn	9.9	10.5	10.7	10.6	0.27	0.05	0.30	0.23
d 89 plasma[3], mg/L								
Zn	1.26	1.28	1.37	1.45	0.047	0.002	0.21	0.42
Cu	0.89	0.92	0.95	0.89	0.036	0.80	0.62	0.11
Fe	2.01	1.86	2.26	1.99	0.115	0.07	0.05	0.56
NEFA[3], mEq/L	130.3	104.9	130.4	105.7	13.58	0.97	0.03	0.98
PUN[4], mg/dL	12.5	9.5	10.8	10.4	0.99	0.61	0.06	0.15

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg DM); SUPZN = CON + 60 mg Zn/kg DM from $ZnSO_4$ + 60 mg Zn/kg DM from Zn-AA complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN).

[2]NO = no supplemental ractopamine hydrochloride; RAC = 300 mg·steer^{-1}·d^{-1} ractopamine hydrochloride (Actogain45; Zoetis, Parsippany, NJ) from day 62 to end of trial.

[3]Plasma trace mineral on d 89; one steer removed from Cu (SUPZN-RAC) and Fe (CON-RAC) analysis.

[4]Serum non-esterified fatty acids on day 89; one steer removed from CON-NO and two steers removed from SUPZN-NO due to lack of sample.

[5]Plasma urea nitrogen on day 89; one steer removed from CON-NO due to lack of sample.

($P = 0.003$) and plasma Fe lesser ($P = 0.05$) for RAC versus NO. Additionally, liver Mn concentrations increased ($P = 0.05$), whereas liver Cu concentrations tended to decrease ($P = 0.07$), in SUPZN compared with CON. No ZNTRT × RACTRT effects were observed for NEFA or PUN ($P \geq 0.15$). However, RAC had lesser NEFA concentrations ($P = 0.03$) and tended to have lesser PUN concentrations ($P = 0.06$) compared with NO. No further effects of ZNTRT or RACTRT were observed for harvest liver, Day 89 plasma trace mineral concentrations, or blood metabolites ($P \geq 0.30$).

Carcass data

Carcass characteristics and carcass-adjusted performance data are reported in Table 5. A ZNTRT × RACTRT effect was observed in DP, BF, and YG

Table 5. Effect of dietary Zn and ractopamine hydrochloride on carcass performance measures

	CON[1]		SUPZN[1]			*P*-value		
Item	NO[2]	RAC[2]	NO[2]	RAC[2]	SEM	ZNTRT	RACTRT	ZNTRT x RACTRT
Steers (*n*)	7	6	7	7				
Carcass characteristics								
HCW, kg[3]	429	438	444	451	7.1	0.05	0.25	0.88
Dress, %	62.0[b]	61.2[b]	61.7[b]	63.4[a]	0.45	0.03	0.28	0.009
KPH, %	2.4	2.3	2.6	2.3	0.12	0.27	0.09	0.43
Back fat, cm	1.60[a]	1.14[b]	1.20[ab]	1.56[ab]	0.161	0.95	0.77	0.01
REA, cm^2	85.9	91.3	96.6	97.1	2.24	0.0008	0.18	0.26
Marbling score4	593	550	544	509	31.3	0.14	0.20	0.91
Yield grade	3.9[a]	3.2[b]	3.1[b]	3.5[ab]	0.21	0.22	0.44	0.02
Carcass-adjusted[5]								
Final BW, kg	691	706	715	726	11.5	0.05	0.26	0.88
Overall ADG, kg/d	2.22	2.38	2.49	2.61	0.128	0.05	0.26	0.88
Overall dry matter intake, kg/d	13.6	13.5	12.9	13.8	0.83	0.84	0.66	0.56
Overall gain to feed	0.165	0.177	0.197	0.194	0.0120	0.04	0.68	0.50

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg DM); SUPZN = CON + 60 mg Zn/kg DM from $ZnSO_4$ + 60 mg Zn/kg DM from Zn-AA complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN).

[2]NO = no supplemental ractopamine hydrochloride; RAC = 300 mg·steer^{-1}·d^{-1} ractopamine hydrochloride (Actogain45; Zoetis, Parsippany, NJ) from day 62 to end of trial.

[3]Initial BW used as covariate in analysis.

[4]Modest = 500-599.

[5]Carcass-adjusted performance was calculated by dividing HCW by the average dressing percentage (62.1%).

[abc]Within a row, means with differing superscripts are different ($P \leq 0.05$).

($P \leq 0.02$). DP was greater in SUPZN-RAC DP than all other treatments. Related to BF, CON-NO presented greater BF than CON-RAC, whereas neither was different from SUPZN-NO or SUPZN-RAC. YG was greater in CON-RAC and SUPZN-NO than CON-NO, where SUPZN-RAC YG was not different from all other treatments. No ZNTRT × RACTRT effects were observed in HCW, KPH, REA, or MS ($P \geq 0.26$). However, steers fed SUPZN had greater REA ($P = 0.0008$) and greater HCW ($P = 0.05$) than CON. A tendency for decreased KPH was observed in RAC steers compared with NO ($P = 0.09$). No further effects of ZNTRT or RACTRT were observed within carcass characteristics ($P \geq 0.14$).

Furthermore, no RACTRT or ZNTRT × RACTRT effects were observed in carcass-adjusted final BW, overall ADG, overall DMI, or overall G:F ($P \geq 0.26$). Steers fed SUPZN had greater carcass-adjusted final BW, overall ADG, and overall G:F ($P \leq 0.04$) compared with CON. However, overall DMI was not influenced by ZNTRT ($P = 0.84$).

Proteomics

Proteomic analysis identified 2,425 proteins. Seventy-five of these proteins differed significantly in abundances (adjusted $P \leq 0.10$) in at least 1 treatment comparison. Thirteen of the 75 identified proteins were also different in at least one treatment comparison when analyzed for phosphorylation modifications. The descriptions of the proteomic analysis of all proteins with significantly different abundances between treatments are reported in Table 6. Fold changes and *P* values of proteins with significantly different abundances between treatments are reported in Tables 7 to 10 and Supplementary Table 1; these include proteins involved in energy metabolism (Table 7), structural proteins (Table 8), protein synthesis/degradation (Table 9), cell health (Table 10), and miscellaneous proteins (Supplementary Table 1).

Supplementation of RAC demonstrates shifts in muscle structure and metabolism proteins, representing potential for greater glycolytic-like muscle fiber characteristics. Supplementation of Zn altered muscle structure proteins, representing potential for greater glycolytic-like muscle fibers. The combination of feeding RAC and Zn influenced proteins related to protein synthesis potentially indicating greater protein synthesis.

Discussion

In previous studies, supplementing Zn at concentrations well above National Academies of Sciences,

Table 6. Descriptions of differentially abundant proteins

Uniprot ID	Protein Name	Sequence Coverage	Unique Peptides	Mascot Score	MW (kDa)	pI
A0A3S5ZPM3	6-phosphogluconate dehydrogenase	10%	3	347	60.7	8.19
P20004	Aconitate hydratase	42%	23	7,141	85.3	7.83
Q3SZR3	Alpha-1-acid glycoprotein	30%	5	98	23.2	5.87
K4JDR8	Alpha-2-macroglobulin	16%	1	80	45.0	7.46
P05631	ATP synthase subunit gamma	14%	2	254	33.1	9.33
A0A3Q1LSB6	ATP-citrate synthase	2%	1	73	120.8	7.24
Q3B7N0	Cadherin-13	5%	3	96	78.1	5.03
A5D7J6	Calreticulin	20%	6	115	48.1	4.48
E1BNE7	Caveolae associated protein 1	17%	6	659	50.2	6.15
Q29RK1	Citrate synthase	35%	12	4,682	51.7	8.12
Q3T0B6	Complement component 1 Q subcomponent binding protein	23%	3	784	30.6	4.86
F6QE33	COP9 signalosome subunit 7A	6%	1	113	30.3	8.24
A0A3Q1LX93	CXXC motif-containing zinc-binding protein	16%	2	26	18.1	5.12
P62894	Cytochrome c	47%	4	1,741	11.7	9.50
O62654	Desmin	26%	9	1,017	53.5	5.27
Q32PH8	Elongation factor 1-alpha 2	59%	19	7,320	50.4	9.03
A6QR19	Enolase 2	25%	2	374,739	47.2	5.07
A5PK37	EPM2A glucan phosphatase	28%	4	173	36.8	6.52
Q6EWQ7	Eukaryotic translation initiation factor 5A-1	52%	7	3,480	16.8	5.24
F1N647	Fatty acid synthase	17%	1	2,748	274.2	6.29
F6QND5	Fibrinogen alpha chain	17%	14	2,709	94.7	6.00
A0A3Q1MG04	Fibrinogen beta chain	26%	10	425	57.4	8.12
A0A3Q1LSN6	Glutathione S-transferase	42%	8	2,212	28.4	6.79
F1MVX2	Glutathione S-transferase LANCL1	33%	8	838	45.3	7.33
A1A4L7	Glutathione S-transferase Mu 1	43%	2	3,316	25.7	7.49
P10096	Glyceraldehyde-3-phosphate dehydrogenase	78%	17	740,918	35.8	8.35
A7MB78	Glycogen synthase	25%	12	2,599	83.8	6.04
F1MCZ0	GMP reductase	6%	1	64	38.0	7.24
Q3ZCH9	Haloacid dehalogenase-like hydrolase domain-containing protein	31%	5	351	28.6	6.65
Q3SZV7	Hemopexin	24%	8	3,171	52.2	7.80
A0A3Q1ND53	Hydroxyacylglutathione hydrolase	14%	6	332	60.5	8.10
O77784	Isocitrate dehydrogenase subunit beta	5%	2	343	42.5	8.68
A0A3Q1M1H0	LIM domain binding 3	23%	10	2,219	66.4	7.99
A0A3Q1M5R4	L-lactate dehydrogenase B	60%	18	22,862	37.4	6.25
Q32LG3	Malate dehydrogenase	66%	18	34,702	35.6	8.54
Q32PA8	Mth938 domain-containing protein	33%	3	1,219	13.4	7.28
A0A1K0FUF3	Myoglobin	95%	31	278,464	17.1	7.46
A4IFM7	Myosin light chain kinase 2	49%	18	8,328	67.2	4.94
Q0P571	Myosin regulatory light chain 2	74%	12	14,743	19.0	5.01
Q29RP6	NAPEPLD protein	21%	6	2,246	43.8	5.67
A4IFQ8	NOL3 protein	17%	2	1,082	22.6	4.17
F1N7X3	Nucleosome assembly protein 1-like 4	5%	2	61	46.4	4.74
Q2HJ33	Obg-like ATPase 1	29%	10	1,391	44.7	7.81
Q5E947	Peroxiredoxin-1	64%	10	5,511	22.2	8.40
P12234	Phosphate carrier protein	10%	4	138	40.1	9.31
A0A3Q1LYW4	Phosphorylase b kinase regulatory subunit	29%	25	6,727	123.1	7.42
A0A3Q1M0J3	Phosphorylase kinase catalytic subunit gamma 1	34%	12	3,365	56.2	7.97
P02584	Profilin-1	65%	7	8,176	15.0	8.28
F1MU19	Proteasome activator complex subunit 2	13%	2	96	28.6	5.27
Q3T0X5	Proteasome subunit alpha type-1	26%	5	151	29.6	6.61
F1N3V8	Protein phosphatase 1 regulatory inhibitor subunit 1A	30%	3	147	17.4	5.58

Table 6. (*Continued*)

Uniprot ID	Protein Name	Sequence Coverage	Unique Peptides	Mascot Score	MW (kDa)	pI
P02638	Protein S100-B	40%	3	216	10.7	4.59
Q3ZC87	Pyruvate kinase	70%	28	244,749	61.4	8.34
P48644	Retinal dehydrogenase 1	27%	11	562	54.8	6.65
G3X6N3	Serotransferrin	64%	40	9,102	77.7	7.17
A0A0A0MP92	Serpin A3-7	6%	3	314	47.0	6.24
A0A3Q1MAU7	S-formylglutathione hydrolase	44%	9	985	32.7	7.65
A0A3Q1LP42	SHSP domain-containing protein	36%	5	150	18.4	5.96
F8SWQ9	Slow skeletal troponin 1	23%	5	933	21.6	9.64
E1BIS6	Synemin	5%	5	93	170.7	4.93
E1BNE2	TIP120 domain-containing protein	24%	17	1,637	134.6	5.63
A5PJM2	TNNI2 protein	30%	6	3,250	23.5	8.98
Q148L6	Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase	27%	7	1,265	36.6	7.30
A0A452DID1	Translationally-controlled tumor protein	10%	2	33	24.6	6.24
O46375	Transthyretin	51%	6	3,451	15.7	6.30
A0A452DIX3	Triosephosphate isomerase	80%	25	114,034	30.6	6.24
P63315	Troponin C, slow skeletal	44%	5	2,166	18.4	4.18
F1N3P2	Ubiquitin carboxyl-terminal hydrolase	6%	3	118	95.8	5.01
A0A3Q1MK32	Ubiquitin-conjugating enzyme E2 L3	52%	6	365	17.9	8.51
A0A3Q1LKR8	Ubiquitin-like modifier-activating enzyme 1	20%	14	839	129.4	5.80
A0A3Q1MT05	UTP—glucose-1-phosphate uridylyltransferase	37%	17	4,486	57.5	7.58
P48616	Vimentin	38%	13	9,189	53.7	5.12
F1N789	Vinculin	33%	29	5,957	123.8	5.76
O97764	Zeta-crystallin	48%	10	978	35.4	8.22

Engineering, and Medicine (2016) recommendations (30 mg Zn/kg DM) has been shown to increase the performance benefits of RAC in beef cattle. Genther-Schroeder et al. (2016a) noted ADG and G:F were linearly increased in steers fed RAC at 300 mg·steer^{-1}·d^{-1} and supplemented a Zn-amino acid complex at 0 to 90 mg Zn/kg DM (88 to 178 mg total dietary Zn/kg DM). Further examination of Zn supplementation (85 or 150 mg dietary Zn/kg DM) to RAC-fed steers indicated greater supplementation of Zn increased ADG and G:F of steers fed RAC at 300 mg·steer^{-1}·d^{-1} for 28 d and increased carcass-adjusted final BW, ADG, and HCW when steers received RAC for 28 or 42 d (Genther-Schroeder et al., 2016b). Interestingly, supranutritional supplementation of Zn (140 mg Zn/kg DM) in the current study improved HCW by 14 kg and improved carcass-adjusted final BW and overall ADG compared with CON steers, regardless of RAC treatment. However, the interaction between ZNTRT and RACTRT for DP is supportive of the synergistic effects of these 2 supplements seen in previous studies (Genther-Schroeder et al., 2016a, 2016b).

Performance responses to Zn and RAC supplementation have been variable. Edenburn et al. (2016) observed no effects of increased Zn supplementation (33 mg Zn/kg DM vs. 97 mg Zn/kg DM) with RAC supplementation (400 mg·steer^{-1}·d^{-1}) on performance or carcass data of steers fed to 638 kg, but steers were only started on the Zn treatment 35 d prior to RAC supplementation. Additionally, Genther-Schroeder et al. (2018) did not see any performance or carcass data effects from steers fed RAC (300 mg·steer^{-1}·d^{-1}) after 47 d of dietary Zn supplementation (total dietary Zn analyzed at 30, 95, 152, 188, 209, or 230 mg Zn/kg DM). Unlike the current study, Genther-Schroeder et al. (2018) utilized steers weighing only 427 kg at the start of the trial and 585 kg at harvest. In comparison, steers in the current study were started on their Zn treatment 62 d prior to RAC supplementation and were approximately 715 kg when harvested. In studies where Zn and RAC had positive effects on performance and carcass data, steers were started on their Zn treatment 42 to 86 d prior to RAC supplementation and were at least 600 kg when harvested. Interestingly, more pronounced beta-adrenergic agonist responses have been observed in heavier cattle (Vestergaard et al., 1994; Winterholler et al., 2007), suggesting greater fat stores provide more substrate for RAC to mobilize and fuel protein accretion. Therefore, the number of days on increased Zn supplementation, as well as the finished BW of the steers being supplemented, may affect the response to RAC.

Table 7. Differentially abundant proteins related to energy metabolism

		Proteomic Data		Phosphorylation Modification	
Uniprot ID	Protein Name	Fold Change	Adj. *P*-value	Fold Change	Adj. *P*-value
CON-RAC/CON-NO[1]					
A0A1K0FUF3	Myoglobin	0.735	< 0.001	–	–
P10096	Glyceraldehyde 3-phosphate dehydrogenase	1.255	0.013	–	–
A0A452DIX3	Triosephosphate isomerase	0.866	0.066	–	–
P20004	Aconitate hydratase	–	–	0.836	0.087
Q148L6	Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase	1.127	0.090	–	–
A0A3S5ZPM3	6-phosphogluconate dehydrogenase	0.886	0.097	–	–
SUPZN-NO/CON-NO[1]					
A0A3Q1M5R4	L-lactate dehydrogenase B	1.175	0.029	–	–
O77784	Isocitrate dehydrogenase subunit beta	0.816	0.056	–	–
F1N647	Fatty acid synthase	–	–	1.331	0.066
Q29RK1	Citrate synthase	0.896	0.082	–	–
A0A1K0FUF3	Myoglobin	0.870	0.085	–	–
SUPZN-RAC/CON-NO[1]					
O77784	Isocitrate dehydrogenase subunit beta	1.249	0.048	–	–
A0A1K0FUF3	Myoglobin	–	–	0.736	0.076
SUPZN-RAC/CON-NO[1]					
A0A3Q1MT05	UTP—glucose-1-phosphate uridylyltransferase	1.107	0.086	–	–
SUPZN-NO/CON-RAC[1]					
F1N647	Fatty acid synthase	1.326	0.004	1.553	0.004
A0A3Q1M5R4	L-lactate dehydrogenase B	1.260	0.010	–	–
P10096	Glyceraldehyde 3-phosphate dehydrogenase	0.807	0.019	–	–
O77784	Isocitrate dehydrogenase subunit beta	0.723	0.028	–	–
A7MB78	Glycogen synthase	–	–	0.763	0.036
Q3ZC87	Pyruvate kinase	0.911	0.088	–	–
P20004	Aconitate hydratase	–	–	1.217	0.089
SUPZN-RAC/CON-RAC[1]					
P20004	Aconitate hydratase	1.141	0.023	–	–
P05631	ATP synthase subunit gamma	1.140	0.044	–	–
SUPZN-RAC/SUPZN-NO[1]					
A7MB78	Glycogen synthase	1.162	0.009	–	–
F1N647	Fatty acid synthase	–	–	0.711	0.035
Q32LG3	Malate dehydrogenase	1.129	0.052	–	–
SUPZN-RAC/SUPZN-NO[1]					
A6QR19	Enolase 2	1.157	0.057	1.595	0.064
P62894	Cytochrome c	1.195	0.059	1.323	0.093
A0A3Q1LSB6	ATP-citrate synthase	0.836	0.082	–	–
P20004	Aconitate hydratase	1.093	0.097	–	–

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg DM); SUPZN = CON + 60 mg Zn/kg DM from $ZnSO_4$ + 60 mg Zn/kg DM from Zn-AA complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN); NO = no supplemental ractopamine hydrochloride; RAC = 300 mg·steer^{-1}·d^{-1} ractopamine hydrochloride (Actogain45; Zoetis, Parsippany, NJ) from day 62 to end of trial.

Structural proteins

In the current study, myosin regulatory light chain 2 abundance was greater in SUPZN-RAC than CON-RAC and CON-NO. As myosin regulatory light chain 2 is increasingly phosphorylated, it is believed to increase muscle fiber contractile force by upregulating the pyrophosphate release of actomyosin crossbridges, even at decreased calcium concentrations (Davis et al., 2002; Bozzo et al., 2003). Myosin light chain kinase 2, the enzyme responsible for phosphorylating myosin regulatory light chain 2, had a greater abundance in RAC-fed steers. This RAC-driven increase in abundance of myosin light chain kinase is also shown in the proteomics data analyzed for phosphorylation modifications. Previous studies have observed an increase in phosphorylated myosin regulatory light chain 2 when the muscle fibers have experienced a slow-to-fast-twitch fiber type shift (Bozzo et al., 2003). It has been hypothesized that fast-twitch

Table 8. Differentially abundant proteins related to muscle structure

		Proteomic Data		Phosphorylation Modification	
Uniprot ID	Protein Name	Fold Change	Adj. *P*-value	Fold Change	Adj. *P*-value
CON-RAC/CON-NO[1]					
F8SWQ9	Slow skeletal troponin 1	0.876	0.045	–	–
P48616	Vimentin	0.860	0.059	–	–
A4IFM7	Myosin light chain kinase 2	–	–	1.236	0.059
SUPZN-NO/CON-NO[1]					
F8SWQ9	Slow skeletal troponin 1	0.891	0.094	–	–
SUPZN-RAC/CON-NO[1]					
A4IFM7	Myosin light chain kinase 2	1.211	0.004	1.477	0.003
Q0P571	Myosin regulatory light chain 2	0.900	0.015	–	–
A5PJM2	TNNI2 protein	0.722	0.052	–	–
A0A3Q1LP42	SHSP domain-containing protein	0.839	0.063	–	–
P48616	Vimentin	0.870	0.086	–	–
E1BIS6	Synemin	1.108	0.087		
SUPZN-NO/CON-RAC[1]					
Q29RP6	NAPEPLD protein	1.140	0.035	–	–
A4IFM7	Myosin light chain kinase 2	0.897	0.046	0.795	0.050
P48616	Vimentin	1.147	0.049	–	–
SUPZN-NO/CON-RAC[1]					
P63315	Troponin C	1.172	0.095	–	–
SUPZN-RAC/CON-RAC[1]					
Q0P571	Myosin regulatory light chain 2	0.864	0.001	–	–
A0A3Q1LP42	SHSP domain-containing protein	0.776	0.004	–	–
Q3B7N0	Cadherin-13	0.864	0.043	–	–
F1N789	Vinculin	0.875	0.054	–	–
SUPZN-RAC/SUPZN-NO[1]					
Q29RP6	NAPEPLD protein	0.843	0.011	–	–
A4IFM7	Myosin light chain kinase 2	1.132	0.022	1.443	0.005
O62654	Desmin	0.859	0.055	–	–
P48616	Vimentin	0.881	0.092	–	–
P02584	Profilin-1	1.091	0.097	–	–

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg DM); SUPZN = CON + 60 mg Zn/kg DM from $ZnSO_4$ + 60 mg Zn/kg DM from Zn-AA complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN); NO = no supplemental ractopamine hydrochloride; RAC = 300 mg·steer^{-1}·d^{-1} ractopamine hydrochloride (Actogain45; Zoetis, Parsippany, NJ) from day 62 to end of trial.

muscle fibers are more responsive to extracellular signals than slow-twitch (Nwoye et al., 1982). This has been especially apparent through beta-adrenergic agonist consistently increasing the cross-sectional area of fast-twitch fibers, resulting in greater muscle mass but having inconsistent effects on slow-twitch fibers (Miller et al., 1988). Additionally, the observed lesser abundance of slow skeletal troponin 1 in both CON-RAC and SUPZN-NO compared with CON-NO supports the myosin light chain data because this protein is more abundant in slow-twitch muscle. Thus, revealing a potential role of both supranutritional Zn and RAC independently influencing muscle fiber characteristics in the biopsies collected in this study. Because histochemical analysis was not conducted in this experiment, it is unknown if these nutritional strategies influenced muscle fiber types.

Energy metabolism

As the proportion of fast-twitch fibers increases, the abundance of glycolytic enzymes would be expected to increase as well. Aconitase is an enzyme in oxidative metabolism responsible for the reversible conversion of citrate to isocitrate. The phosphorylation of mitochondrial aconitase results in an increase in the reverse reaction (Lin et al., 2009). This decreases the amount of substrate entering the electron transport chain and results in decreased mitochondrial function and impaired oxidative metabolism. In the present study, aconitase was more abundant in SUPZN-RAC compared with CON-RAC or SUPZN-NO, and abundance was greater in SUPZN-NO and CON-NO compared with CON-RAC when analyzed for phosphorylation modifications. Therefore, Zn and RAC

Table 9. Differentially abundant proteins related to protein synthesis/degradation pathways

		Proteomic Data		Phosphorylation Modification	
Uniprot ID	Protein Name	Fold Change	Adj. *P*-value	Fold Change	Adj. *P*-value
CON-RAC/CON-NO[1]					
Q3T0X5	Proteasome subunit alpha type-1	1.177	0.022	–	–
A0A452DID1	Translationally-controlled tumor protein	–	–	1.749	0.033
SUPZN-NO/CON-NO[1]					
F6QE33	COP9 signalosome subunit 7A	1.282	0.051	–	–
SUPZN-RAC/CON-NO[1]					
A0A452DID1	Translationally-controlled tumor protein	–	–	1.860	0.010
Q32PH8	Elongation factor 1-alpha 2	1.103	0.018	–	–
F1N3P2	Ubiquitin carboxyl-terminal hydrolase	1.215	0.033	–	–
F6QE33	COP9 signalosome subunit 7A	1.344	0.034	–	–
Q6EWQ7	Eukaryotic translation initiation factor 5A-1	–	–	1.355	0.036
SUPZN-NO/CON-RAC[1]					
O97764	Zeta-crystallin	1.193	0.034	–	–
E1BNE2	TIP120 domain-containing protein	1.167	0.046	–	–
A0A3Q1MK32	Ubiquitin-conjugating enzyme E2 L3	0.875	0.068	–	–
SUPZN-RAC/CON-RAC[1]					
F1MU19	Proteasome activator complex subunit 2	1.208	0.024	–	–
A0A3Q1LKR8	Ubiquitin-like modifier-activating enzyme 1	0.833	0.064	–	–
Q3T0X5	Proteasome subunit alpha type-1	0.897	0.097	–	–
SUPZN-RAC/SUPZN-NO[1]					
A0A3Q1MK32	Ubiquitin-conjugating enzyme E2 L3	1.168	0.038	–	–
E1BNE7	Caveolae associated protein 1	0.906	0.079	–	–
Q6EWQ7	Eukaryotic translation initiation factor 5A-1	1.112	0.099	1.342	0.047
A0A452DID1	Translationally-controlled tumor protein	1.188	0.099	–	–

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg DM); SUPZN = CON + 60 mg Zn/kg DM from $ZnSO_4$ + 60 mg Zn/kg DM from Zn-AA complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN); NO = no supplemental ractopamine hydrochloride; RAC = 300 mg·steer^{-1}·d^{-1} ractopamine hydrochloride (Actogain45; Zoetis, Parsippany, NJ) from day 62 to end of trial.

may independently be facilitating the phosphorylation of aconitase to reduce the oxidative potential of muscle fiber characteristics. L-lactate dehydrogenase B is a Zn-dependent enzyme (Price, 1962) responsible for the anaerobic conversion of pyruvate to lactate and is more abundant in glycolytic fibers (Huber et al., 2007). In the current study, L-lactate dehydrogenase B had an increased abundance in SUPZN-NO compared with CON-NO and CON-RAC. This may suggest that steers fed SUPZN had greater ability to utilize lactate as an energy substrate, an effect that carried over into postmortem muscle and hastened pH decline as well as postmortem tenderization as reported by Schulte et al. (2021). Interestingly, this effect was diminished by RAC supplementation, resulting in a slower fall in postmortem pH and greater time needed for postmortem tenderization (Schulte et al., 2021). In addition, the abundance of the aerobic enzyme citrate synthase was lesser in SUPZN-NO compared with CON-NO, showing the decreased oxidative metabolic potential in SUPZN-NO. Myoglobin is more abundant in oxidative fibers because of the need to transport oxygen to facilitate greater aerobic metabolism. Myoglobin was greater in abundance in CON-NO compared with CON-RAC and SUPZN-NO and was also greater in abundance in CON-NO compared with SUPZN-RAC when analyzed for phosphorylation modifications. These differences were also confirmed in muscle at 1 h postmortem, wherein CON-NO–fed steers had a greater abundance of myoglobin compared with CON-RAC–fed animals (Schulte, unpublished data, 2023). This difference could relate to muscle fiber characteristics; however, muscle fiber types were not analyzed in the current study.

Protein synthesis/degradation

Along with metabolic shifts, in the current study, both supranutritional Zn and RAC affected the abundance of enzymes involved in protein synthesis in the *longissimus thoracis*. Supplementation of RAC resulted in increased abundance of eukaryotic initiation factor 5A in SUPZN-RAC compared with SUPZN-NO as well as an increased abundance in SUPZN-RAC compared with SUPZN-NO and CON-NO when

Table 10. Differentially abundant proteins related to cellular health

		Proteomic Data		Phosphorylation Modification	
Uniprot ID	Protein Name	Fold Change	Adj. *P*-value	Fold Change	Adj. *P*-value
CON-RAC/CON-NO[1]					
Q3SZR3	Alpha-1-acid glycoprotein	1.251	0.023	–	–
SUPZN-NO/CON-NO[1]					
A0A3Q1MG04	Fibrinogen beta chain	1.252	0.049	–	–
A1A4L7	Glutathione S-transferase mu 1	0.726	0.075	–	–
F6QND5	Fibrinogen alpha chain	1.176	0.079	–	–
SUPZN-RAC/CON-NO[1]					
A0A3Q1MG04	Fibrinogen beta chain	–	–	0.657	0.003
F1MVX2	Glutathione S-transferase LANCL1	0.862	0.047	–	–
Q5E947	Peroxiredoxin-1	1.141	0.047	–	–
SUPZN-NO/CON-RAC[1]					
A0A3Q1LSN6	Glutathione S-transferase	1.347	0.047	–	–
A1A4L7	Glutathione S-transferase mu 1	0.518	0.058	–	–
A0A3Q1MG04	Fibrinogen beta chain	1.236	0.066	–	–
SUPZN-RAC/CON-RAC[1]					
Q3SZR3	Alpha-1-acid glycoprotein	0.717	0.001	–	–
A0A3Q1MAU7	S-formylglutathione hydrolase	1.143	0.086	–	–
SUPZN-RAC/SUPZN-NO[1]					
A0A3Q1MG04	Fibrinogen beta chain	0.772	0.017	0.561	0.005
A0A3Q1ND53	Hydroxyacylglutathione hydrolase	1.182	0.090	–	–
F6QND5	Fibrinogen alpha chain	0.848	0.095	–	–

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg DM); SUPZN = CON + 60 mg Zn/kg DM from $ZnSO_4$ + 60 mg Zn/kg DM from Zn-AA complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN); NO = no supplemental ractopamine hydrochloride; RAC = 300 mg·steer^{-1}·d^{-1} ractopamine hydrochloride (Actogain45; Zoetis, Parsippany, NJ) from day 62 to end of trial.

analyzed for phosphorylation modifications. This specific initiation factor is present in differentiating satellite cells (Luchessi et al., 2009), and muscle fibers are able to synthesize protein at only 70% of the typical rate when the fibers are depleted of this initiation factor (Kang and Hershey, 1994). Although the role of phosphorylation on this factor has yet to be defined, its positive regulation of translation has been observed when it is phosphorylated (Chung et al., 2013). Additionally, elongation factor 1-alpha 2 was more abundant in SUPZN-RAC compared with CON-NO. This factor is needed for the elongation of proteins in the ribosome during protein synthesis (Sanges et al., 2012). The greater abundance of both of these factors in SUPZN-RAC demonstrates the increased potential for protein synthesis because of combined effect of Zn and RAC fed together.

These Zn effects on enzymes involved in protein synthesis paired with greater HCW in SUPZN steers suggest that increased Zn supplementation is critical to improve cattle growth. In the current study, Zn supplementation resulted in a 10.92% increase in plasma Zn concentrations by Day 62 that continued throughout the RAC feeding period. Similarly, Genther-Schroeder et al. (2016a) observed a linear increase in plasma Zn concentrations following 78 d of Zn supplementation (60, 90, 120, or 150 mg Zn/kg DM) prior to feeding RAC. This plasma Zn response corresponded to linear improvements in growth performance during RAC supplementation (Genther-Schroeder et al., 2016a), suggesting an increased pool of circulating Zn may be critical to support RAC-induced growth processes. Furthermore, both SUPZN and RAC increased liver Zn concentrations, even though liver Zn is readily difficult to change (Suttle, 2010). However, a linear decrease in liver Zn concentrations with increasing Zn supplementation within RAC-fed steers observed by Genther-Schroeder et al. (2016a) contradicts the findings of the current study. Perhaps supplemental Zn was great enough to support increased circulating Zn concentrations and Zn storage in the liver. However, more research is warranted to understand how supplemental Zn influences liver and plasma Zn concentrations and how body Zn stores affect the growth performance of RAC-fed steers.

Cell health proteins

Both Zn and Fe have been shown to be sequestered during infection to prevent their use by infectious

Supplementary Table 1. Miscellaneous differentially abundant proteins

		Proteomic Data		Phosphorylation Modification	
Uniprot ID	Protein Name	Fold Change	Adj. *P*-value	Fold Change	Adj. *P*-value
CON-RAC/CON-NO[1]					
A0A3Q1LX93	CXXC motif-containing zinc-binding protein	0.835	0.030	–	–
A5D7J6	Calreticulin	0.838	0.073	–	–
SUPZN-NO/CON-NO[1]					
A4IFQ8	NOL3 protein	0.890	0.007	–	–
Q2HJ33	Obg-like ATPase 1	0.837	0.008	–	–
A0A3Q1LX93	CXXC motif-containing zinc-binding protein	0.809	0.010	–	–
A0A3Q1LYW4	Phosphorylase b kinase regulatory subunit	0.896	0.038	–	–
Q3T0B6	Complement component 1 Q subcomponent binding protein	0.811	0.040	–	–
P48644	Retinal dehydrogenase 1	1.227	0.053	–	–
Q3ZCH9	Haloacid dehalogenase-like hydrolase domain-containing protein	1.248	0.071	–	–
F1MCZ0	GMP reductase	0.829	0.078	–	–
A0A0A0MP92	Serpin A3-7	0.822	0.081	–	–
K4JDR8	Alpha-2-macroglobulin	1.161	0.088	–	–
O46375	Transthyretin	–	–	1.344	0.10
SUPZN-RAC/CON-NO[1]					
A0A3Q1LX93	CXXC motif-containing zinc-binding protein	0.826	0.033	–	–
A5PK37	EPM2A glucan phosphatase	1.247	0.035	–	–
P12234	Phosphate carrier protein	1.245	0.053	–	–
A0A3Q1M0J3	Phosphorylase kinase catalytic subunit gamma 1	1.135	0.076	1.381	0.042
G3X6N3	Serotransferrin	0.898	0.082	–	–
Q3SZV7	Hemopexin	–	–	1.244	0.079
O46375	Transthyretin	0.805	0.085	–	–
SUPZN-NO/CON-RAC[1]					
Q3SZV7	Hemopexin	1.140	0.008	–	–
Q32PA8	Mth938 domain-containing protein	0.894	0.047	–	–
O46375	Transthyretin	1.195	0.051	1.443	0.058
F1MCZ0	GMP reductase	0.760	0.055	–	–
A0A3Q1M1H0	LIM domain binding 3	1.149	0.063	–	–
Q3ZCH9	Haloacid dehalogenase-like hydrolase domain-containing protein	1.189	0.074	–	–
SUPZN-RAC/CON-RAC[1]					
A0A3Q1M0J3	Phosphorylase kinase catalytic subunit gamma 1	1.140	0.074	1.319	0.080
Q3SZV7	Hemopexin	1.129	0.078	1.227	0.071
SUPZN-RAC/SUPZN-NO[1]					
A0A3Q1M1H0	LIM domain binding 3	0.845	0.017	–	–
O46375	Transthyretin	0.733	0.017	0.700	0.061
Q32PA8	Mth938 domain-containing protein	1.106	0.037	–	–
F1N7X3	Nucleosome assembly protein 1-like 4	1.181	0.051	–	–
A5PK37	EPM2A glucan phosphatase	1.191	0.097	–	–
A0A3Q1M0J3	Phosphorylase kinase catalytic subunit gamma 1	–	–	1.299	0.100

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg DM); SUPZN = CON + 60 mg Zn/kg DM from $ZnSO_4$ + 60 mg Zn/kg DM from Zn-AA complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN); NO = no supplemental ractopamine hydrochloride; RAC = 300 mg·steer^{-1}·d^{-1} ractopamine hydrochloride (Actogain45; Zoetis, Parsippany, NJ) from day 62 to end of trial.

microbes in a process known as nutritional immunity (Corbin et al., 2008; Kehl-Fie and Skaar, 2010). Genther-Schroeder et al. (2016b) hypothesized supplementation of RAC may be causing an inflammatory response, leading to an increase in Zn and Fe in the liver. Although not statistically significant in the study by Genther-Schroeder et al. (2016b) or the current study, this RAC effect of increased liver Zn and Fe appears to be numerically driven by increased supplementation of Zn. Genther-Schroeder et al. (2016b) did not measure liver Fe, but RAC was shown to decrease plasma Fe similarly to the decrease in plasma Fe observed in the current study. These data further support the hypothesis that Fe is being sequestered.

In addition to supranutritional Zn supplementation influencing liver and plasma Fe concentrations, the current study observed ZNTRT effects on liver Mn and Cu concentrations. Increased liver Mn concentrations at harvest caused by SUPZN appear to be driven by the numerically decreased liver Mn in CON-NO steers. However, a similar effect was observed by Carmichael et al. (2018) when Mn absorption and retention were increased with increasing Zn supplementation. Perhaps this effect is in response to Mn and Zn competing for Zrt/Irt-like proteins (ZIP) transporters to enter the liver (Lin et al., 2017). Consistent with our understanding of Zn's antagonistic pressure on Cu (Reeves et al., 1993; Kincaid, 2000), SUPZN steers tended to have 14.41% lower liver Cu concentrations at harvest than CON steers. Similarly, Messersmith et al. (2021b) observed a 6.52% decrease in liver Cu concentrations in heifers supplemented 100 vs. 30 mg Zn/kg DM for 168 d. Stable plasma Cu concentrations throughout the study suggest the antagonism between Zn and Cu more strongly influences liver Cu stores than circulating Cu concentrations. Furthermore, liver Cu concentrations of Zn supplemented steers remained adequate (Kincaid, 2000), indicating the antagonism was not severe.

Miscellaneous proteins

RAC has been shown to decrease lipogenesis and increase lipolysis (Mills and Liu, 1990; Peterla and Scanes, 1990; Ross et al. 2011). Lesser abundance of fatty acid synthase in the *longissimus thoracis* of CON-RAC steers versus SUPZN-NO steers in the present study may reflect the depressive effect of RAC on lipogenic enzymes. The current study also noted decreased serum NEFA concentrations caused by RAC, consistent with observations by Messersmith et al. (2021a). This effect is likely a result of rapidly growing tissues picking up NEFA from the bloodstream as an energy source at a faster rate than they are being released. It has been established that RAC supplementation increases N retention as protein is accreted in the animal (Ricke et al., 1999; Ross et al., 2011; Carmichael et al., 2018). As a result, less muscle protein is likely to be catabolized, supporting the lesser PUN noted in RAC steers in the present study.

Conclusions

In conclusion, supranutritional Zn and RAC supplementation positively affect carcass-adjusted performance. Because of the lack of positive effects of Zn supplementation when it is initiated close to the start of RAC supplementation (Edenburn et al., 2016), it is interesting to speculate that there may be a minimum number of days of Zn supplementation needed prior to RAC supplementation. Although muscle fiber type was not measured in the current study, supranutritional Zn and RAC supplementation, both independently and collectively, demonstrates evidence of an altered muscle fiber characteristics through shifting structural and metabolic enzyme abundances and phosphorylation states. More research is needed to determine if Zn supplementation is shifting muscle fiber type and, if so, how this occurs in order to design a Zn and RAC supplementation strategy to maximize these hypertrophic effects.

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