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Carcass Characteristics and Quality Attributes of Beef From Cattle Supplemented Zinc and Ractopamine Hydrochloride

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Abstract: The objective of this experiment was to identify the impact of supranutritional zinc (SUPZN) and ractopamine hydrochloride (RAC) supplementation of beef steers on postmortem protein degradation and tenderness development of extended (>14 d) aged steaks. It was hypothesized that RAC and SUPZN supplementation would influence meat quality development during extended aging of the *longissimus thoracis* muscle. Crossbred steers ($n = 27$) were fed in a 2×2 factorial: control (CON; analyzed 36 mg Zn/kg dry matter) or supranutritional Zn supplementation (SUPZN; CON diet + 60 mg Zn/kg dry matter [from $ZnSO_4$] + 60 mg Zn/kg dry matter [from Zn–amino acid complex]) dietary treatments for the entire 91-d trial. Before harvest (30 d), steers were blocked by body weight within Zn treatments to RAC treatments of 0 (NO) or 300 (RAC) mg per steer per day. Steers were harvested at a commercial processing facility. Carcass characteristics were collected 2 d postmortem. Warner-Bratzler shear force value (7, 14, 28, and 42 d postmortem), calpain-1 autolysis (2 d postmortem), and desmin degradation (2, 7, 14, 28, and 42 d postmortem) were analyzed. RAC supplementation resulted in greater ($P < 0.02$) Warner-Bratzler shear force values at 7, 14, and 28 d postmortem, lesser ($P < 0.01$) calpain-1 autolysis (76-kDa band) at 2 d postmortem, and lesser ($P < 0.02$) desmin degradation at 2, 7, 14, and 28 d postmortem. Supplementation of Zn resulted in greater ($P < 0.01$) calpain-1 autolysis (78- and 76-kDa band) at 2 d postmortem and a trend for greater ($P = 0.08$) desmin degradation at 2 d postmortem. Tougher steaks from RAC-supplemented steers were explained by slowed postmortem proteolysis, and Zn supplementation showed evidence of enhanced proteolysis early postmortem (2 d). These results demonstrate that nutritional supplementation can impact the rate of tenderness development and postmortem proteolysis in muscle.

Key words: beef quality, extended aging, proteolysis, tenderness, ractopamine hydrochloride, zinc
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Introduction

Fresh beef tenderness development is one of the most important traits influencing consumer acceptance of beef products (Wilfong et al., 2016). Although tenderness is an important purchasing trait, consumers are also increasingly concerned about the sustainability of beef production (de Souza et al., 2017). Nutritional supplementation strategies, such as the use of the beta-agonists ractopamine hydrochloride (RAC) and the trace mineral zinc (Zn) above the nutritional recommendations (National Academy of Sciences, Engineering, and Medicine, 2015), can positively impact the growth and efficiency of beef cattle (Boler et al., 2012; Lean et al., 2014; Genther-Schroeder et al., 2016a), thus influencing beef production sustainability (Lawrence and Ibarburu, 2005; Johnson et al., 2013). However, some studies have shown no benefit of Zn supplementation (Bohrer et al., 2014). This lack of benefit of Zn supplementation could depend on the Zn source, dosage, and duration of feeding.

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Supplementation of RAC and Zn in combination can influence early postmortem (Schulte et al., 2021) and aged beef tenderness values (Boler et al., 2012; Bohrer et al., 2014; Vellini et al., 2020). It has previously been identified that aging past 21 d postmortem was necessary to diminish the negative impacts of RAC supplementation on tenderness (Boler et al., 2012). Increased toughness in steaks from RAC supplemented (200 to 300 mg per animal) and supplementation of other beta-agonists is attributed to decreased proteolysis due to increased calpastatin activity (Strydom et al., 2009; Cruz et al., 2021). Early postmortem decreases in tenderness values in steaks from RAC supplemented cattle were explained by a slower rate of postmortem protein degradation in muscle from cattle supplemented RAC (Schulte et al., 2021).

Zinc-only supplementation has been shown to have tendencies to improve tenderness values early (1 d; $P = 0.06$) postmortem (Schulte et al., 2021) in Angus steers and later (28 d; $P = 0.07$) postmortem in Nellore bulls (Vellini et al., 2020). However, when Zn is fed in combination with RAC, these trends for improved tenderness values due to Zn supplementation do not remain (Bohrer et al., 2014; Paulk et al., 2014; Edenburn et al., 2016; Schulte et al., 2021). In the study by Schulte et al. (2021), improved tenderness at 1 d postmortem in steaks from steers supplemented Zn only (as Zn amino acid complex and Zn sulfate) was attributed to a lesser pH value at 6 h postmortem ($P = 0.06$), resulting in lower Warner-Bratzler shear force (WBSF) values from Zn-only–supplemented steers. Tougher steaks at 1 d from steers supplemented RAC had a slower pH decline and needed additional days of aging to tenderize (Schulte et al., 2021). Vellini et al. (2020) proposed that Zn supplementation (as Zn amino acid complex and Zn chromium) may be impacting the proteolytic process. This impact of Zn supplementation on tenderness development needs to be further studied to define the impacts on the proteolytic system of postmortem muscle.

It was hypothesized that Zn and RAC supplementation would influence tenderness development of extended (>14 d) aged *longissimus thoracis* muscle through alterations in proteolytic mechanisms. The objective of this experiment was to characterize how Zn and RAC supplementation of beef steers affects postmortem protein degradation and tenderness development of *longissimus thoracis* steaks throughout aging.

Materials and Methods

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

Sample collection

Crossbred Angus steers ($n = 28$; 7 steers per treatment; ~431 kg initial body weight) were obtained from a single source. Steers were fed one of 4 diets in GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). Individual feed intake was recorded on each steer, and thus each steer was an experimental unit. Steers were fed the same basal diet. Steers were balanced across Zn and RAC treatments based on genetic growth potential, as analyzed by Gene Max (GeneMax Focus, Zoetis, Parsippany, NJ) gain scores and steer initial body weights. Zn treatments consisted of a control (CON; analyzed 36 mg Zn/kg dry matter) that is similar to the National Research Council recommendation (National Academy of Sciences, Engineering, and Medicine, 2015) or supranutritional Zn supplementation (SUPZN; CON diet + 60 mg Zn/kg dry matter [$ZnSO_4$] + 60 mg/kg dry matter as zinc amino acid complex [Availa-Zn; Zinpro, Eden Prairie, MN]). Zn supplementation occurred for the entire 91-d feeding trial. RAC supplementation occurred for the last 30 d before harvest. RAC treatments consisted of 0 mg (NO) or 300 mg (RAC) of RAC (Actogain45, Zoetis, Parsippany, NJ) per steer per day. Nutritional treatments (CON-NO, SUPZN-NO, and SUPZN-RAC) had 7 steers except for CON-RAC, which had 6 steers complete the trial (1 steer was removed from all analysis due to illness not related to the treatment). At the end of the feeding period, final body weights (~739 kg ± 11.3–12.2 kg) were collected, and steers were transported and harvested at a commercial processing facility (Tama, IA) on a single day. A muscle sample (approximately 200 g) was collected at 48 h postmortem from the right side of the carcass and stored (−80°C) for laboratory analysis. Carcass characteristics (hot carcass weight, area of the *longissimus* muscle, 12th rib backfat thickness, kidney, pelvic, and heart fat, and marbling score) were collected from the right side of the carcass by trained United States Department of Agriculture and university personnel at 48 h postmortem. The dressing percentage of each carcass was calculated using the following equation: hot carcass weight in kg/live weight × 100. Yield grade values were calculated using the following equation: 2.5 + (2.5 × fat thickness [in]) + (0.0038 × hot carcass

weight [lb]) + (0.2 × kidney, pelvic, and heart fat [%]) − (0.32 × area of the *longissimus* muscle [in^2]). Whole rib sections (Institutional Meat Purchase Specifications #112) (USDA, 2014) from the left side of the carcass were collected at 3 d postmortem and transported to the Iowa State University Meat Lab (Ames, IA) for steak fabrication at 6 d postmortem. Steaks containing the *longissimus thoracis* muscle were fabricated into nine 2.54-cm-thick and nine 0.64-cm-thick steaks (Figure 1).

The first 2.54-cm-thick steak of each sample was denuded of all exterior fat and connective tissue with only the *longissimus thoracis* muscle remaining and frozen (6 d postmortem) until prepared for composition analysis as described by Schulte et al. (2021) using a CEM ORACLE System (AOAC International, 2013; CEM Corporation, Matthews, NC) for fat analysis, CEM SMART 6 System (AOAC International, 2013; CEM Corporation) for moisture analysis, and CEM Sprint Rapid Protein Analyzer (AOAC, 2011; CEM Corporation) for protein analysis.

The remaining eight 2.54-cm-thick steaks were utilized for quality data collection (percentage purge, pH, marbling score, percentage cook loss, *L**, *a**, *b**, and WBSF), and the nine 0.64-cm-thick steaks were utilized for laboratory analysis (calpain-1 and desmin degradation). Steaks were vacuum packaged, aged (2°C) for 7 d (1 additional d after fabrication), 14 d, 28 d, or 42 d postmortem, and frozen (−29°C) until quality or laboratory analysis (Figure 1). Pairs of steaks for quality analysis were thawed (24 h at 2°C) for collection of the following data as described by Schulte et al. (2021): percentage purge, pH, Hunter *L**, *a**, and *b** values, marbling score, cook loss, and WBSF values. Percentage purge was calculated: [(the weight of the package with purge − the weight of the package without purge)/(the steak weight in the package with purge − the weight of the package without purge)] × 100. A Hanna HI9025 pH meter (Hanna Instruments, Woonsocket, RI) was utilized to collect pH values at each day of aging post freezing. The pH meter was calibrated using pH 4 and 7 buffers. The accuracy range of 6.95 to 7.05 in the pH 7 buffer was monitored after every 5th measurement. After 30 min of bloom time at room temperature (~22°C), a HunterLab MiniScan EZ 4500L colorimeter (Hunter Associates Laboratory Inc., Reston, VA) was used to collect surface color measurements (*L**, *a**, and *b** values) using a 10° observer angle, illuminant D65 (daylight at 6,500 K), and 2.4-cm aperture. USDA marbling scores were determined by two trained personnel (USDA, 2017). Steaks were cooked to an internal temperature of 68°C on clamshell grills (Cuisinart, East Windsor, NJ), and temperatures were monitored using thermocouples. Cook loss was calculated: [(raw steak weight −cooked steak weight)/raw steak weight] × 100. At least 3 cores (1.27-cm-thick) per steak were removed parallel to the muscle fiber for WBSF analysis (American Meat Science Association, 2016). A minimum of 6 cores per postmortem timepoint per carcass was utilized. An Instron (Model 5566, Instron Corporations, Norwood, MA) was fitted with a WBSF attachment (100 kg compression load cell and crosshead

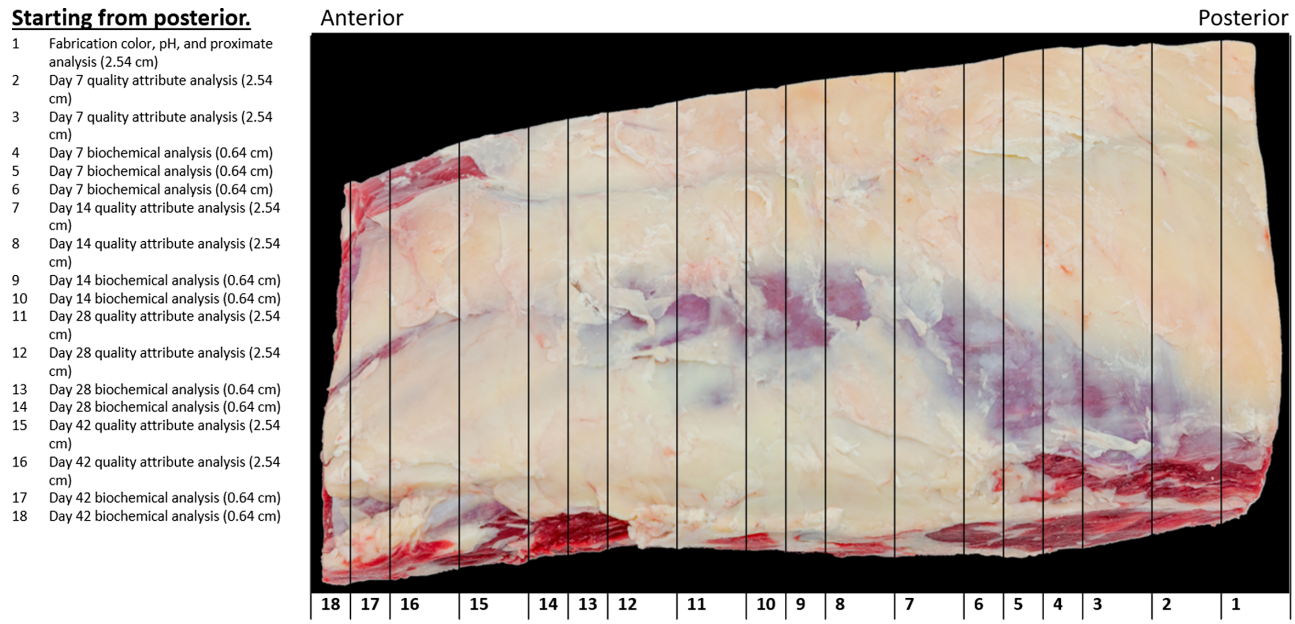

Figure 1. Steak fabrication layout for quality attribute and biochemical analysis.

speed of 3.3 mm/s) for instrumental tenderness analysis.

Muscle protein extraction

Frozen meat (200 g) was homogenized and powdered in liquid nitrogen. Samples from each postmortem timepoint (0.5 g) were homogenized, and muscle proteins were solubilized in 10 mM sodium phosphate (pH 7.0) and 2% sodium dodecyl sulfate (SDS) (wt/vol) as described by Carlson et al. (2017).

Western blots: Desmin and calpain-1

Desmin degradation (2, 7, 14, 28, and 42 d postmortem) and calpain-1 autolysis (2 d postmortem) were determined on whole muscle protein extracts. One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted as Carlson et al. (2017) described. A reference sample (a mixture of 4 representative samples from the current study at 2 and 42 d postmortem containing intact and degradation bands) was created for whole muscle desmin degradation analysis. Another sample (mixture of 4 representative samples from the current study at 2 d postmortem containing 80-, 78-, and 76-kDa calpain-1 bands) was created for whole muscle calpain-1 autolysis analysis. The mixed 2- and 42-d reference sample was used for desmin blot analysis. The 2 d mixed reference was used on each calpain-1 blot to indicate the calpain-1 80-, 78-, and 76-kDa bands. The representative reference samples were loaded on each gel for western blot analysis. For desmin degradation, 15% SDS-PAGE gels (10 cm × 10 cm; acrylamide: N, N'-bis-methylene acrylamide = 100:1 [wt/wt], 0.1% [wt/vol] SDS, 0.05% [vol/vol] tetramethylene diamine, 0.05% [wt/vol] ammonium persulfate, 0.5 M Tris-HCl [pH 8.8]) were used. For calpain-1 autolysis, 10% SDS-PAGE gels (10 cm × 10 cm; acrylamide: N, N'-bis-methylene acrylamide = 100:1 [wt/wt], 0.1% [wt/vol] SDS, 0.05% [vol/vol] tetramethylene diamine, 0.05% [wt/vol] ammonium persulfate, 0.5 M Tris-HCl [pH 8.8]) were used. Protein (40 µg for desmin and calpain-1 analysis) was loaded into gel well. All gels were run on SE 260 Hoefer Mighty Small II electrophoresis units (Hoefer, Inc., Holliston, MA). Desmin gels were run for approximately 360 volt-hours, and calpain-1 gels were run for approximately 320 volt-hours. After running, the SDS-PAGE gels were transferred to polyvinylidene difluoride membranes (0.2 µm pore size, Immobilon-PSQ, 26.5 by 3.75 M RL, VCAT# ISEQ00010, Millipore Corporation, Billerica, MA) as described by Carlson et al. (2017). Western blotting was conducted as described by Carlson et al. (2017). Briefly, membranes were blocked in phosphate-buffered saline (PBS)-Tween mixed with 5% nonfat dry milk, incubated for 1 h at room temperature (22° C), and then poured off. Primary antibody concentrations were diluted in PBS-Tween and added to each blot for incubation overnight (4°C) as follows: desmin (1:40,000) using polyclonal rabbit anti-desmin antibody produced at Iowa State University (Huff-Lonergan et al., 1996); calpain-1 (1:5,000) using monoclonal mouse anti-calpain-1 (MA3-940; Thermo-Scientific, Waltham, MA). After overnight incubation with primary antibodies, blots were washed 3 times in PBS-Tween for 10 min intervals. Secondary antibodies were prepared in PBS-Tween at the following concentrations: desmin secondary (1:20,000): goat anti-rabbit horseradish peroxidase (HRP) antibody (32430; Thermo-Scientific); calpain-1 secondary (1:10,000): goat anti-mouse HRP antibody (A2554; Sigma Aldrich, St. Louis, MO) with 5% nonfat dry milk. Blots were incubated in secondary antibodies for 1 h at room temperature (22°C). After secondary antibody incubation, blots were washed 3 times in PBS-Tween for 10 min intervals. Blots were imaged as described by Carlson et al. (2017). The intensity of the 38-kDa desmin degradation product was quantified as a ratio of the sample band to the internal reference (2 and 42 d mix) of each gel. Calpain-1 autolysis was analyzed as a percentage of the 80-, 78-, or 76-kDa bands within each sample. All western blots were completed in at least duplicate with a coefficient of variance value of the band of interest of 20% or less.

Mineral analysis

Muscle samples at 7 d postmortem were homogenized and powdered in liquid nitrogen. Analysis of trace minerals (Zn, copper, iron) was conducted using 0.5 g of powdered muscle sample as described by Pogge and Hansen (2013).

Statistical analysis

Data were analyzed using the Mixed procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC) as a 2 × 2 factorial with fixed effects of Zn and RAC and the interaction. Initial body weight was used as a covariate for hot carcass weight analysis. Hot carcass weight was used as a covariate to analyze ribeye area, fat thickness, kidney, pelvic, and heart fat, and yield grade. Whole muscle desmin degradation and calpain-1 autolysis data were analyzed using the Mixed procedure of SAS (version 9.4, SAS Institute Inc.) as a 2 × 2 factorial with fixed effects of Zn and RAC

and the interaction; gel was used as a random effect in the models. Significance levels for all analyses were set at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. The least-squares means, and standard errors, were reported for all measured attributes. The least-squares means were separated using SAS's PDIFF procedure (version 9.4; SAS Institute Inc.).

Results and Discussion

Carcass and quality characteristics data

Supplementation of RAC and Zn is utilized in the beef industry to improve growth performance and carcass yields (Boler et al., 2012; Bohrer et al., 2014; Genther-Schroeder et al., 2016a; Hagenmaier et al., 2017). In the current study, there was a significant interaction of feeding Zn and RAC in that steers fed supranutritional Zn and RAC in combination had a greater dressing percentage ($P = 0.01$; Table 1) compared with steers not fed Zn and RAC in combination. The interaction of feeding supranutritional Zn and RAC resulted in greater 12th rib fat thickness ($P = 0.02$; Table 1) and greater yield grades ($P = 0.02$; Table 1) compared with steers fed Zn or RAC separately.

Supplementation of supranutritional Zn resulted in greater hot carcass weights ($P = 0.05$) and larger ribeye areas ($P < 0.01$) regardless of RAC supplementation (Table 1). The impact of feeding RAC and Zn, separately and in combination in the literature, has been inconsistent with impacts on carcass characteristics. Some studies have demonstrated improvements in carcass characteristics of beef cattle supplemented RAC and Zn (Boler et al., 2012; Bohrer et al., 2014; Genther-Schroeder et al., 2016a; Genther-Schroeder et al., 2016b; Van Bibber-Krueger et al., 2017;

Table 1. Effect of supranutritional zinc (Zn) and ractopamine hydrochloride (RAC) supplementation on carcass characteristics and proximate composition of beef finishing steers *longissimus thoracis* muscle

	CON[1]		SUPZN[1]		*P* Value		
Item	NO[2]	RAC[2]	NO[2]	RAC[2]	ZNTRT	RACTRT	ZNTRT × RACTRT
Steers (*n*)	7	6	7	7			
Initial Body Weight, kg	514 ± 9.3	512 ± 10.0	508 ± 9.3	516 ± 9.3	0.95	0.77	0.58
Live Weight, kg	723 ± 11.3	747 ± 12.2	751 ± 11.3	742 ± 11.3	0.34	0.53	0.16
Hot Carcass Weight, kg	430 ± 6.6	439 ± 7.1	445 ± 6.7	452 ± 6.6	0.05	0.26	0.88
Dressing Percentage	59.5 ± 0.4[b]	58.8 ± 0.4[b]	59.3 ± 0.4[b]	60.9 ± 0.4[a]	0.03	0.28	0.01
Ribeye Area, cm^2	86.9 ± 2.1	91.8 ± 2.2	96.4 ± 2.0	96.0 ± 2.1	< 0.01	0.31	0.21
12th Rib Fat Thickness, cm	1.6 ± 0.2[a]	1.2 ± 0.2[b]	1.2 ± 0.2[ab]	1.5 ± 0.2[ab]	0.83	0.65	0.02
Kidney, pelvic, and heart fat, %	2.4 ± 0.1	2.3 ± 0.1	2.6 ± 0.1	2.3 ± 0.1	0.41	0.08	0.42
Yield Grade[3]	4.0 ± 0.2[a]	3.2 ± 0.2[b]	3.1 ± 0.2[b]	3.4 ± 0.2[ab]	0.11	0.29	0.02
Marbling Score[4]	610 ± 30	550 ± 30	540 ± 30	500 ± 30	0.06	0.11	0.88
Protein, %[5]	22.5 ± 0.3	22.8 ± 0.4	22.7 ± 0.3	23.2 ± 0.3	0.38	0.30	0.72
Fat, %[5]	7.9 ± 0.7	6.4 ± 0.7	6.6 ± 0.7	6.9 ± 0.7	0.55	0.42	0.19
Moisture, %[5]	69.7 ± 0.4	70.8 ± 0.5	70.6 ± 0.4	70.2 ± 0.4	0.75	0.51	0.10
Mineral Content							
Copper, mg/kg DM[6]	1.3 ± 0.4	0.8 ± 0.4	2.2 ± 0.4	1.2 ± 0.4	0.12	0.08	0.50
Iron, mg/kg DM[6]	52 ± 2.9	52 ± 3.13	53 ± 2.9	50 ± 2.9	0.95	0.61	0.71
Zinc, mg/kg DM[6]	121 ± 6.0[ab]	125 ± 6.5[ab]	136 ± 6.0[a]	113 ± 6.0[b]	0.85	0.13	0.03

[a,b]Means with different superscripts within rows are significantly different ($P \leq 0.05$).

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg dry matter); SUPZN = CON + 60 mg Zn/kg dry matter from $ZnSO_4$ + 60 mg Zn/kg dry matter from Zn-amino acid complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN). Fed for the entire 89 d trial.

[2]NO = no supplemental RAC; RAC = 300 mg RAC per head per day (Actogain45; Zoetis, Parsippany, NJ) starting 28 d before harvest.

[3]Yield Grade were calculated using the following equation: 2.5+(2.5 * back fat thickness [in]) + (0.0038 * hot carcass weight [lb]) + (0.2 * kidney, pelvic, and heart fat [%]) −(0.32 * ribeye area [in^2]).

[4]Marbling Scores: 400 = small; 500 = modest.

[5]Measured as a percentage of protein, fat, and moisture measurements.

[6]DM = dry matter.

CON-NO = No Zn or RAC supplementation. CON-RAC = No Zn supplementation, only RAC supplantation. SUPZN-NO = Zn supplementation, no RAC supplementation. SUPZN-RAC = Zn and RAC supplementation.

Vellini et al., 2020), whereas others have demonstrated no differences in carcass characteristics of beef cattle supplemented RAC and Zn (Edenburn et al., 2016; Genther-Schroeder et al., 2018).

In a study of feedlot heifers (Van Bibber-Krueger et al., 2017), heifers supplemented Zn (analyzed 131.90 mg Zn/kg dry matter; 43 d trial) as Zn sulfate in combination with 200 mg RAC for the final 42 d before harvest had a lesser yield grade than heifers fed Zn (analyzed 131.90 mg Zn/kg dry matter) without RAC supplementation. Van Bibber-Krueger et al. (2017) also saw a trend for a lesser ($P = 0.08$) dressing percentage in heifers supplemented a lesser amount of Zn (analyzed 61.90 mg Zn/kg dry matter) in combination with RAC than those fed greater Zn (analyzed 131.90 mg Zn/kg dry matter) in combination with RAC. Genther-Schroeder et al. (2016a) showed no difference in 12th rib backfat, yield grade, or dressing percentage in steers supplemented Zn (analyzed 150 mg Zn/kg dry matter; 84- to 91-d trial) as Zn amino acid complex and Zn sulfate and RAC (300 mg per steer per day for the final 28 d before harvest) compared with non-supplemented steers (no supplemented RAC and analyzed 85 mg Zn/kg dry matter). Edenburn et al. (2016) also showed no difference in 12th rib backfat, yield grade, or dressing percentage in steers supplemented RAC (400 mg per head per day; 28 d before harvest) and Zn (analyzed 100.98 mg Zn/kg dry matter; 63 d on trial) as Zn-propionate compared with non-supplemented steers. Lastly, Bohrer et al. (2014) also saw no differences in these carcass characteristics of steers fed RAC (300 mg per day per steer for the final 35 d before harvest) and supplemental Zn (analyzed 159.73 mg Zn/kg dry matter; 63 d on treatment) as Zn propionate. These studies differ from the current study (Table 1), which saw a greater dressing percentage, 12th rib back fat, and yield grade in steers supplemented Zn (analyzed 140 mg Zn/kg dry matter) as Zn amino acid complex and Zn sulfate in combination with RAC (300 mg per head per day for the final 28 d before harvest) compared with steers supplemented Zn or RAC separately. These studies show the complexity of feeding RAC and Zn in combination related to carcass characteristics and need to further investigate optimal feeding conditions (dosage, length of dosage, form of Zn fed).

In the current study, all cattle that were supplemented supranutritional Zn, regardless of RAC feeding, resulted in greater hot carcass weights ($P = 0.05$) and ribeye areas ($P < 0.01$; Table 1). The Zn-only treatment had a 6 kg and 15 kg advantage in hot carcass weight over the 2 treatment groups without Zn supplementation (CON-RAC and CON-NO treatments, respectively; Table 1). A greater advantage in hot carcass weight was seen in the combination treatment (SUPZN-RAC), which had a 13 kg and 22 kg advantage in hot carcass weight over the CON-RAC and CON-NO treatments, respectively (Table 1). The Zn-only treatment had a larger ribeye area (Table 1) than CON-RAC (4.6 cm^2 difference) and CON-NO (9.5 cm^2 difference). The combination treatment (SUPZN-RAC) had a larger ribeye area (Table 1) than CON-RAC (4.2 cm^2 difference) and CON-NO (9.1 cm^2 difference). Vellini et al. (2020) supplemented Zn (analyzed 108 mg Zn/kg dry matter; 95 d on trial) as Zn amino acid alone or in combination with chromium methionine to Nellore bulls and saw a trend for greater ($P = 0.08$) *longissimus* muscle area in bulls fed Zn and chromium methionine compared with bulls fed Zn only or no supplementation. This improvement in the *longissimus* muscle area may have been due to Zn and chromium supplementation (Vellini et al., 2020). These Nellore bulls did not differ in hot carcass weights (Vellini et al., 2020). Genther-Schroeder et al. (2016b) showed no difference in hot carcass weight and ribeye area of steers fed Zn only (analyzed 118, 148, or 178 mg Zn/kg dry matter) compared with steers fed no supplemental Zn or supplemental Zn in combination with RAC (300 mg per head per day for 28 d before harvest) supplementation. In a study analyzing 3 different sources of Zn (Zn-sulfate, -methionine, -propionate) supplemented at 75 mg Zn/kg dry matter in addition to the basal (44.1-52.5 mg Zn/kg dry matter) diet (Nunnery et al., 2007), researchers observed no differences in hot carcass weight or ribeye area of heifers supplemented Zn compared with those not supplemented Zn. These heifers were on feed for 154 to 182 d depending on a visual appraisal of finish (Nunnery et al., 2007). Van Bibber-Krueger et al. (2017) saw a decrease in ribeye area when heifers were fed Zn (analyzed 131.90 mg Zn/kg dry matter; 43 d trial) as Zn sulfate compared with 30 mg Zn (analyzed 61.90 mg Zn/kg dry matter) supplemented as Zn sulfate, but no difference in hot carcass weight between Zn supplementation rates was observed. These inconsistencies in Zn supplementation on hot carcass weight and ribeye area between trials are likely due to differences in Zn dosage, dosage duration, finished animal weight, and genetic variability. Further investigation into understanding optimal Zn supplementation will help to define consistent results.

Muscle mineral analysis

The interaction of feeding Zn and RAC in combination significantly impacted muscle Zn content with

lesser Zn in the muscle of cattle fed the combination of Zn and RAC compared with cattle not supplemented Zn and RAC in combination ($P = 0.03$; Table 1). Increased muscle Zn in SUPZN-NO suggests these steers had greater tissue Zn available to support Zn-dependent processes, which perhaps was decreased by increased demands of growth (greater dressing percentage) in SUPZN-RAC. Others have shown that RAC decreases liver Zn, but the relationship between liver and muscle Zn has not been evaluated (Genther-Schroeder et al., 2016b).

Warner-Bratzler shear force values

The interaction of feeding Zn and RAC in combination was not significant at any postmortem time point for WBSF values.

Supplementation of RAC can negatively influence the WBSF values of steaks from the longissimus (Avendaño-Reyes et al., 2006; Scramlin et al., 2010; Boler et al., 2012; Bohrer et al., 2014; Lean et al., 2014; Schulte et al., 2021). Supplementation of Zn can result in decreased *longissimus* steak WBSF values early (1 d; $P = 0.06$) postmortem (Schulte et al., 2021) and later (28 d; $P = 0.07$) postmortem (Vellini et al., 2020). If Zn supplementation occurs in combination with RAC supplementation, these potential positive impacts on WBSF values are negated (Bohrer et al., 2014; Edenburn et al., 2016; Schulte et al., 2021). In the current study, greater WBSF values were observed in steaks from steers supplemented RAC, regardless of feeding Zn in combination, at 7 d ($P = 0.02$), 14 d ($P = 0.01$), and 28 d ($P = 0.01$) d postmortem compared with steaks from steers not supplemented RAC (Figure 2). This difference did not remain at 42 d postmortem ($P = 0.78$; Figure 2). The supplementation of RAC consistently results in greater WBSF values in beef *longissimus lumborum* muscle, typically from 1 to 21 d postmortem, compared with muscle from cattle not supplemented RAC (Avendaño-Reyes et al., 2006; Scramlin et al., 2010; Boler et al., 2012; Bohrer et al., 2014; Lean et al., 2014; Schulte et al., 2021). This consistently greater WBSF value has been demonstrated early (1 d) postmortem (Schulte et al., 2021) as well as later (3, 7, 14, and 28 d) postmortem (Scramlin et al., 2010; Boler et al., 2012; Bohrer et al., 2014). This increased WBSF value is likely due to the impacts of RAC supplementation on the calpain proteolytic system and postmortem protein degradation.

Supplementation of Zn did not result in significant differences in WBSF values at any postmortem timepoint (Figure 2). Although the main effect of Zn was not significant for WBSF analysis, the Zn-only treatment had a numerically lesser WBSF value at 7, 14,

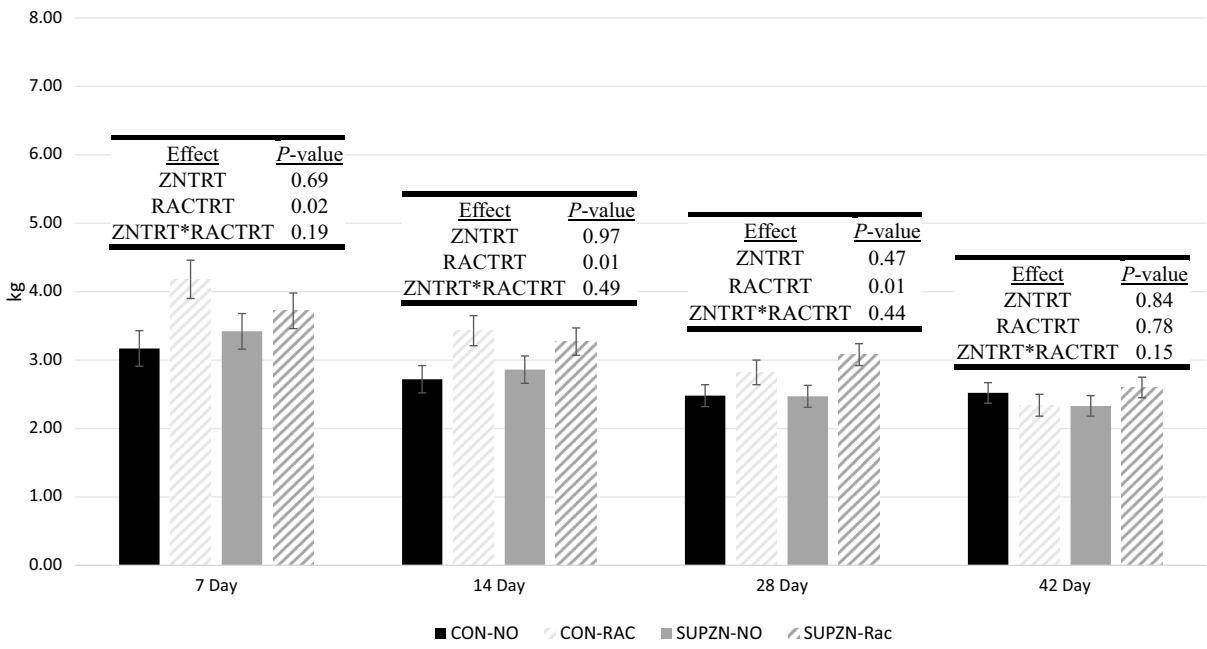

Figure 2. Effect of supranutritional zinc (Zn) and ractopamine hydrochloride (RAC) supplementation on Warner-Bratzler shear force (WBSF) values of beef *longissimus thoracis* steaks at different postmortem timepoints. [1]CON = no supplemental Zn (analyzed 36 mg Zn/kg dry matter); SUPZN = CON + 60 mg Zn/kg dry matter from $ZnSO_4$ + 60 mg Zn/kg dry matter from Zn-amino acid complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN). Fed for the entire 89 d trial. [2]NO = no supplemental RAC; RAC = 300 mg RAC per head per d (Actogain45; Zoetis, Parsippany, NJ) starting 28 d before harvest. [3]WBSF values were averaged across two adjacent steaks.

and 28 d postmortem than either RAC supplemented treatment. The Zn-only treatment had a 0.76, 0.57, and 0.35 lesser kg WBSF value than the RAC-only treatment at 7, 14, and 28 d postmortem, respectively. Compared with the combination treatment (SUPZN-RAC), the Zn-only treatment had a 0.30, 0.41, and 0.61 kg lesser WBSF value at 7, 14, and 28 d postmortem. This numerically lesser WBSF value is like the study by Schulte et al. (2021), in which steaks from Zn-only supplemented cattle had a lesser WBSF value at 1 d postmortem than steaks from either RAC-supplemented treatment (CON-RAC and SUPZN-RAC). The study by Schulte et al. (2021), combined with the current study's numerically lesser WBSF values in the Zn-only–supplemented treatment, demonstrates a potential for more rapid achievement of lesser WBSF values in beef from Zn-only–supplemented steers compared with steaks from steers supplemented RAC. The persistence of the observation that Zn tends to affect the rate of tenderization is intriguing, and the molecular explanation should be investigated.

The current study had limited differences in aged steak quality characteristics (percentage purge, pH, marbling score, percentage cook loss, L^*, a^*, b^*, hue angle, and chroma values). These data can be found in Supplemental Table 1.

Postmortem protein degradation

The interaction of feeding Zn and RAC in combination resulted in lesser desmin degradation at 2 d postmortem (Table 2).

The calpain proteolytic system is the primary protease system responsible for postmortem protein degradation in meat that impacts the postmortem tenderness development (Geesink et al., 2006; Koohmaraie and Geesink, 2006). Calpain-1 is the primary isoform responsible for early postmortem protein degradation, as evidenced by limited proteolysis in calpain-1 knockout mice (Geesink et al., 2006). Calpain-1 requires micromolar concentrations of calcium for activation (Suzuki et al., 1981; Goll et al., 2003), and once activated, the 80-kDa catalytic subunit will autolyze the intermediate 78-kDa and fully autolyzed 76-kDa subunits. Once fully autolyzed, calpain-1 loses its activity (Goll et al., 2003). Although calpain activity is lost, the 76-kDa autolyzed subunit indicates that calpain-1 was active, and thus postmortem protein degradation occurred. The inhibitor of the calpains, calpastatin, plays an integral part in preventing protein degradation, thus impacting postmortem tenderness development. Previously in Brangus bulls and steers, the rate of postmortem tenderization was linked to calpastatin activity

Table 2. Effect of supranutritional zinc (Zn) and ractopamine hydrochloride (RAC) supplementation on whole muscle extract calpain-1 autolysis and desmin degradation of beef *longissimus thoracis* muscle at different postmortem timepoints

	CON[1]		SUPZN[1]		*P* Value		
Item	NO[2]	RAC[2]	NO[2]	RAC[2]	ZNTRT	RACTRT	ZNTRT × RACTRT
Calpain Autolysis, 2 Days Postmortem[3]							
80 kDa[4]	22.3 ± 1.4	26.5 ± 1.6	22.2 ± 1.3	25.00 ± 1.3	0.50	< 0.01	0.52
78 kDa[4]	37.4 ± 1.1	38.8 ± 1.2	34.1 ± 1.1	36.60 ± 1.1	0.01	0.04	0.55
76 kDa[4]	41.5 ± 1.3	36.6 ± 1.3	44.4 ± 1.5	39.33 ± 1.4	0.04	< 0.01	0.93
Desmin Degradation[5]							
2 Days Postmortem	0.20 ± 0.07[b]	0.12 ± 0.07[b]	0.47 ± 0.07[a]	0.11 ± 0.07[b]	0.08	< 0.01	0.05
7 Days Postmortem	1.09 ± 0.23	0.71 ± 0.25	1.40 ± 0.23	0.77 ± 0.23	0.40	0.02	0.56
14 Days Postmortem	2.18 ± 0.26	1.30 ± 0.30	2.17 ± 0.27	1.16 ± 0.27	0.77	< 0.01	0.81
28 Days Postmortem	2.10 ± 0.36	1.40 ± 0.37	2.06 ± 0.38	1.10 ± 0.36	0.53	< 0.01	0.63
42 Days Postmortem	2.77 ± 0.21	2.11 ± 0.22	2.11 ± 0.22	1.92 ± 0.24	0.06	0.06	0.30

[a,b]Means with different superscripts within rows are significantly different ($P \leq 0.05$).

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg dry matter); SUPZN = CON + 60 mg Zn/kg dry matter from $ZnSO_4$ + 60 mg Zn/kg dry matter from Zn-amino acid complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN). Fed for the entire 89 d trial.

[2]NO = no supplemental RAC; RAC = 300 mg RAC per head per day (Actogain45; Zoetis, Parsippany, NJ) starting 28 d before harvest.

[3]Whole muscle extracted calpain-1.

[4]Values are expressed as a ratio of the catalytic subunit present as the intact band (80 kDa) or the autolyzed bands (78 and 76 kDa) of the catalytic subunit of calpain-1.

[5]Ratio of the densitometry units of the degraded 38-kDa band of the sample compared with the 38-kDa band of the reference sample.

CON-NO = No Zn or RAC supplementation. CON-RAC= No Zn supplementation, only RAC supplantation. SUPZN-NO = Zn supplementation, no RAC supplementation. SUPZN-RAC = Zn and RAC supplementation.

and postmortem protein degradation of troponin-T (Lonergan et al., 2001). Lonergan et al. (2001) showed that a faster tenderization rate in the steaks from Brangus steers than steaks from Brangus bulls was explained by lesser calpastatin activity and greater troponin-T degradation. In a subset of samples from their study sorted by WBSF values (Lonergan et al., 2001), more tender samples (measured with WBSF values) at 2 and 7 d postmortem were negatively correlated with calpastatin activity, suggesting a relationship between calpastatin to the rate and extent of postmortem tenderization.

Increased toughness in meat from cattle supplemented RAC is typically attributed to increased calpastatin activity (Strydom et al., 2009; Cruz et al., 2021), decreasing calpain activity and protein degradation (Lonergan et al., 2001). A study of pork *longissimus dorsi* muscle has shown decreased calpain-1 activity in muscle from pigs fed RAC without observing differences in calpastatin activity (Sainz et al., 1993); thus, investigating multiple aspects of the calpain proteolytic system is necessary. In the current study, a greater WBSF value at 7, 14, and 28 d postmortem was observed in muscle from cattle supplemented RAC ($P \leq 0.02$; Figure 2) and explained by lesser ($P < 0.01$) autolyzed (76-kDa) and greater 80-kDa ($P < 0.01$) and 78-kDa ($P = 0.04$) unautolyzed calpain-1 at 2 d postmortem compared with muscle from cattle not supplemented RAC (Table 2; Figure 3). Decreased postmortem protein degradation in muscle from RAC-fed steers was further demonstrated by lesser desmin degradation at 7 d ($P = 0.02$), 14 d ($P < 0.01$), and 28 d ($P < 0.01$) postmortem (Table 2; Figure 3) in muscle from cattle supplemented RAC than cattle not supplemented RAC. In the study by Boler et al. (2012), cattle supplemented RAC (300 mg per steer per day for the final 28 d before harvest) had greater WBSF values at 4, 7, 14, and 21 d postmortem. These differences were small (< 0.60 kg difference) but continued unless aged past 21 d postmortem (Boler et al., 2012). Decreased tenderness in RAC supplemented steers (Boler et al., 2012) was most likely attributed to reduced proteolysis. However, protein degradation, calpain autolysis, and calpastatin activity were not measured by Boler et al. (2012). In the current study, differences in WBSF values were numerically a kg or less, and differences decreased across days of aging (Figure 2), but once again, consistently greater WBSF values existed in steers supplemented RAC. Although calpastatin activity was not measured in the current study, the reduction in calpain-1 76-kDa autolysis and desmin degradation lend credence to the negative impact of RAC supplementation on postmortem proteolysis existing in the current study.

In the current study, muscle from cattle supplemented supranutritional Zn had a greater ($P = 0.04$) percentage of autolyzed (76-kDa) and lesser ($P = 0.01$) abundance of partially autolyzed (78-kDa) calpain-1 at 2 d postmortem than cattle not supplemented Zn (Table 2). Although the interaction of feeding Zn and RAC in combination was significant at 2 d postmortem to result in lesser desmin degradation, supplementation of supranutritional Zn-only showed trends of greater desmin degradation at 2 d postmortem (Table 2). The Zn-only treatment (SUPZN-NO) had 4 times greater (0.48; Table 2) desmin degradation than either RAC-supplemented treatment (CON-RAC, 0.12; SUPZN-RAC, 0.11). Compared with the (CON-NO) treatment, SUPZN-NO had over 2 times the ratio of the control (0.20) at 2 d postmortem (Table 2). This numerically greater ratio of desmin degradation products continued until 14 d postmortem compared with the control treatment (CON-NO) and 42 d postmortem compared with the RAC-supplemented treatments (Table 2). Although these differences in calpain-1 autolysis and desmin degradation did not result in different WBSF values at any postmortem timepoint (Figure 2), differences in Zn and RAC supplementation

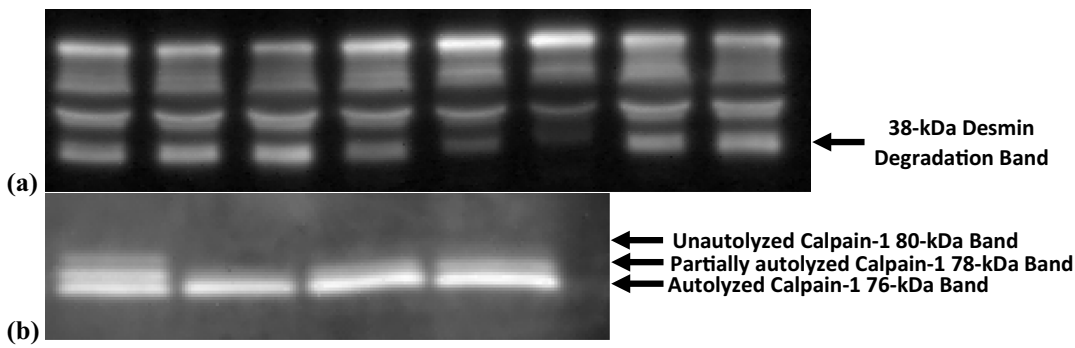

Figure 3. Representative western blots of whole muscle desmin (a) and calpain-1 (b).

have been reported previously to impact tenderness (Schulte et al., 2021). Schulte et al. (2021) demonstrated that muscle from cattle fed the same supranutritional Zn treatment as the current study had a trend for a lesser ($P = 0.06$) WBSF value at 1 d postmortem than muscle from cattle not supplemented supranutritional Zn. Muscle from cattle fed with RAC had a significantly ($P < 0.01$) higher WBSF value at 1 d postmortem than muscle from cattle not fed RAC. The difference in WBSF values between cattle supplemented RAC only and Zn only was 2.2 kg at 1 d postmortem (Schulte et al., 2021). In their study (Schulte et al., 2021), Zn supplementation trended for a lower ($P = 0.06$) pH value at 6 h postmortem that was linked to the trend for lower ($P = 0.06$) WBSF values at 1 d postmortem. The greater WBSF values in the RAC-supplemented cattle were attributed to lesser desmin and troponin-T degradation detected in the whole muscle extracts of steaks from RAC supplemented cattle (Schulte et al., 2021). In the current study, cattle were harvested in a commercial facility, and early postmortem (1 h and 1 d) pH and muscle samples were not obtained. However, the data in the current study show similar trends to the study by Schulte et al. (2021), in which muscle from RAC-supplemented steers had lesser protein degradation and greater WBSF values early postmortem. In the study by Schulte et al. (2021), the muscle from the Zn-only–supplemented beef had a trend for lower ($P = 0.06$) pH values at 6 h postmortem, lesser ($P = 0.06$) WBSF values at 1 d postmortem, and numerically greater troponin-T degradation at 1 d postmortem than muscle from cattle not supplemented Zn. Seeing this impact of supranutritional Zn supplementation in the current and previous studies (Schulte et al., 2021) demonstrates the potential of Zn supplementation to influence the rate at which postmortem protein degradation and tenderness development occur, most likely explained by postmortem pH decline and metabolism. Further investigation into the role of Zn and how it can impact postmortem metabolism, and thus potentially dictate tenderness development, is necessary.

Conclusions

Supplementation of supranutritional Zn to steers resulted in an earlier onset of proteolysis shown by greater calpain-1 autolysis and numerically greater desmin degradation at 2 d postmortem. However, this only resulted in numerically lesser WBSF values of steaks at 7 d postmortem from steers supplemented supranutritional Zn compared with steers not supplemented supranutritional Zn. Samples from steers supplemented Zn also had greater ribeye areas and heavier hot carcass weights than steers not supplemented Zn. Supplementation of RAC resulted in increased toughness unless aged over 28 d postmortem, which was explained by lesser desmin degradation and calpain-1 autolysis. The data support the conclusion that supplementation of supranutritional Zn-only shows signs of a potential feeding practice to enhance the rate of postmortem proteolysis and potentially hasten the rate of tenderness development.

Acknowledgments

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Literature Cited

American Meat Science Association. 2016. AMSA guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. Version 1.02. Am. Meat Sci. Assoc. Champaign, IL.

AOAC International. 2011. Protein in raw and processed meats. Official method 2011.04. In: Official methods of analysis. AOAC Int., Gaithersburg, MD.

AOAC International. 2013. Moisture and fat in meats microwave and nuclear magnetic resonance analysis. Official method 2008.06. In: Official methods of analysis. AOAC Int., Gaithersburg, MD.

Avendaño-Reyes, L., V. Torres-Rodríguez, F. J. Meraz-Murillo, C. Perez-Linares, F. Figueroa-Saavedra, and P. H. Robinson. 2006. Effects of two β-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. J. Anim. Sci. 84:3259–3265. https://doi.org/10.2527/jas.2006-173.

Bohrer, B. M., B. M. Edenburn, D. D. Boler, A. C. Digler, and T. L. Felix. 2014. Effects of feeding ractopamine hydrochloride (Optaflexx) with or without supplemental zinc and chromium propionate on growth performance, carcass characteristics, and meat quality of finishing steers. J. Anim. Sci. 92:3988–3996. https://doi.org/10.2527/jas.2015-9979.

Boler, D. D., A. L. Shreck, D. B. Faulkner, J. Killefer, F. K. McKeith, J. W. Homm, and J. A. Scanga. 2012. Effect of ractopamine hydrochloride (Optaflexx) dose on live animal performance, carcass characteristics and tenderness in early weaned beef steers. Meat Sci. 92:458–463. https://doi.org/10.1016/j.meatsci.2012.05.011.

Carlson, K. B., K. J. Prusa, C. A. Fedler, E. M. Steadham, A. C. Outhouse, D. A. King, E. Huff-Lonergan, and S. M.

Lonergan. 2017. Postmortem protein degradation is a key contributor to fresh pork loin tenderness. J. Anim. Sci. 95:1574–1586. https://doi.org/10.2527/jas2016.1032.

Cruz, F. L., E. B. de Carvalho, E. M. Ramos, L. J. Pereira, and M. G. Zangeronimo. 2021. Relationship between beta-adrenergic agonists, calpain system activity and beef texture: A systematic review. J. Anim. Physiol. An. N. 105:442–451. https://doi.org/10.1111/jpn.13479.

de Souza, D. M., R. Petre, F. Jackson, M. Hadarits, S. Pogue, C. N. Carlyle, E. Bork, and T. McAllister. 2017. A review of sustainability enhancements in the beef value chain: State-of-the-art and recommendations for future improvements. Animals. 7:1–8. https://doi.org/10.3390/ani7030026.

Edenburn, B. M., S. G. Kneeskern, B. M. Bohrer, W. Rounds, D. D. Boler, A. C. Dilger, and T. L. Felix. 2016. Effects of supplementing zinc or chromium to finishing steers fed ractopamine hydrochloride on growth performance, carcass characteristics, and meat quality. J. Anim. Sci. 94:771–779. https://doi.org/10.2527/jas.2015-9979.

Geesink, G. H., S. Kuchay, A. H. Chishti, and M. Koohmaraie. 2006. μ-calpain is essential for postmortem proteolysis of muscle proteins. J. Anim. Sci. 84:2834–2840. https://doi.org/10.2527/jas.2006-122.

Genther-Schroeder, O. N., M. E. Branine, and S. L. Hansen. 2016a. The influence of supplemental Zn-amino acid complex and ractopamine hydrochloride feeding duration on growth performance and carcass characteristics of finishing beef cattle. J. Anim. Sci. 94:4338–4345. https://doi.org/10.2527/jas.2015-0159.

Genther-Schroeder, O. N., M. E. Branine, and S. L. Hansen. 2016b. The effects of increasing supplementation of zinc-amino acid complex on growth performance, carcass characteristics, and inflammatory response of beef cattle fed ractopamine hydrochloride. J. Anim. Sci. 94:3389–3398. https://doi.org/10.2527/jas2015-0209.

Genther-Schroeder, O. N., M. E. Branine, and S. L. Hansen. 2018. Effects of increasing supplemental dietary Zn concentration on growth performance and carcass characteristics in finishing steers fed ractopamine hydrochloride. J. Anim. Sci. 96:1903–1913. https://doi.org/10.1093/jas/sky094.

Goll, D. E., V. F. Thompson, H. Li, W. Wei, and J. Cong. 2003. The calpain system. Physiol. Rev. 83:731–801. https://doi.org/10.1152/physrev.00029.2002.

Hagenmaier, J. A., C. D. Reinhardt, M. J. Ritter, M. S. Calvo-Lorenzo, G. J. Vogel, C. A. Guthrie, M. G. Siemen, K. F. Lechtenberg, D. J. Rezac, and D. U. Thomson. 2017. Effects of ractopamine hydrochloride on growth performance, carcass characteristics, and physiological response to different handling techniques. J. Anim. Sci. 95:1977–1992. https://doi.org/10.2527/jas2016.0936.

Huff-Lonergan, E., T. Mitsuhashi, D. D. Beekman, F. C. Parrish, D. G. Olson, and R. M. Robson. 1996. Proteolysis of specific muscle structural proteins by μ-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. J. Anim. Sci. 74:993–1008. https://doi.org/10.2527/1996.745993x.

Johnson, B. J., F. R. B. Ribeiro, and J. L. Beckett. 2013. Application of growth technologies in enhancing food security and sustainability. Animal Frontiers 3:8–13. https://doi.org/10.2527/af.2013-0018.

Koohmaraie, M., and G. H. Geesink. 2006. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Sci. 74:34–43. https://doi.org/10.1016/j.meatsci.2006.04.025.

Lawrence, J. D., and M. A. Ibarburu. 2005. Economic analysis of pharmaceutical technologies in modern beef production. Iowa State University. p. 1–28. https://ageconsearch.umn.edu/record/37560 (Accessed 17 March 2022).

Lean, I. J., J. M. Thompson, and F. R. Dunshea. 2014. Metaanalysis of zilpaterol and ractopamine effects of feedlot performance, carcass traits and shear strength of meat in cattle. PLoS ONE 9:e115904. https://doi.org/10.1371/journal.pone.0115904.

Lonergan, S. M., E. Huff-Lonergan, B. R. Wiegand, and L. A. Kriese-Anderson. 2001. Postmortem proteolysis and tenderization of top loin steaks from Brangus cattle. J. Muscle Foods. 12:121–136. https://doi.org/10.1111/j.1745-4573.2001.tb00304.x.

National Academy of Sciences, Engineering, and Medicine. 2015. Nutrient requirements of beef cattle: 8th revised edition. The National Academies Press, Washington, D.C.

Nunnery, G. A., J. T. Vasconcelos, C. H. Parsons, G. B. Salyer, P. J. Defoor, F. R. Valdez, and M. L. Galyean. 2007. Effects of source of supplemental zinc on performance and humoral immunity in beef heifers. J. Anim. Sci. 85:2304–2313. https://doi.org/10.2527/jas.2007-0167.

Paulk, C. B., M. D. Tokach, J. L. Nelssen, D. D. Burnett, M. A. Vaughn, K. J. Phelps, S. S. Dritz, J. M. DeRouchey, R. D. Goodband, J. C. Woodworth, T. A. Houser, K. D. Haydon, and J. M. Gonzalez. 2014. Effect of dietary zinc and ractopamine hydrochloride on pork chop muscle fiber type distribution, tenderness, and color characteristics. J. Anim. Sci. 92:2325–2335. https://doi.org.10.2527/jas.2013-7318.

Pogge, D. J., and S. L. Hansen. 2013. Supplemental vitamin C improves marbling in feedlot cattle consuming high sulfur diets. J. Anim. Sci. 91:4303–4314. https://doi.org.10.2527/jas.2012-5638.

Sainz, R., Y. Kim, F. Dunshea, and R. Campbell. 1993. Effects of ractopamine in pig muscles: histology, calpains and β-adrenergic receptors. Aust. J. Agr. Res. 44:1441–1448. https://doi.org/10.1071/ar9931441.

Schulte, M. D., K. G. Hochmuth, E. M. Steadham, K. J. Prusa, S. M. Lonergan, S. L. Hansen, and E. Huff-Lonergan. 2021. The influence of supranutritional zinc and ractopamine hydrochloride supplementation on early postmortem pH decline and meat quality development of beef. Meat Muscle Biol. 5:1–15. https://doi.org/10.22175/mmb.12250.

Scramlin, S. M., W. J. Platter, R. A. Gomez, W. T. Choat, F. K. McKeith, and J. Killefer. 2010. Comparative effects of ractopamine hydrochloride and zilpaterol hydrochloride on growth performance, carcass traits, and longissimus tenderness of finishing steers. J. Anim. Sci. 88:1823–1829. https://doi.org/10.2527/jas.2009-2405.

Strydom, P. E., L. Frylinck, J. L. Montgomery, and M. F. Smith. 2009. The comparison of three β-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. Meat Sci. 81:557–564. https://doi.org/10.1016/j.meatsci.2008.10.011.

Suzuki, K., S. Tsuji, S. Ishiura, Y. Kimura, S. Kubota, and K. Imahori. 1981. Autolysis of calcium-activated neutral protease of chicken skeletal muscle. J. Biochem. 90: 1787–1793. https://doi.org/10.1093/oxfordjournals.jbchem.a133656.

USDA. 2014. Institutional Meat Purchase Specifications: Fresh Beef Series 100. US Department of Agriculture. 1–46. https://www.ams.usda.gov/sites/default/files/media/IMPS_100_Fresh_Beef%5B1%5D.pdf. (Accessed 10 May 2022).

USDA. 2017. Standards for Grades of Carcass Beef. US Department of Agriculture. 1–16. https://www.ams.usda.gov/sites/default/files/media/CarcassBeefStandard.pdf. (Accessed 12 April 2022).

Van Bibber-Krueger, C. L., R. G. Amachawadi, H. M. Scott, J. M. Gonzalez, and J. S. Drouillard. 2017. Interactive effects of supplemental Zn sulfate and ractopamine hydrochloride on growth performance, carcass traits, and plasma urea nitrogen in feedlot heifers. J. Anim. Sci. 95:4638–4645. https://doi.org/10.2527/jas2017.1764.

Vellini, B. L., L. F. Prados, F. P. Monção, A. K. Fireman, F. D. Resende, and G. R. Siqueira. 2020. Zinc amino acid complex in association with chromium methionine improves the feed efficiency of finished Nellore cattle in the feedlot. Anim. Feed Sci. Tech. 262:1–12. https://doi.org/10.1016/j.anifeedsci.2020.114430.

Wilfong, A. K., K. V. McKillip, J. M. Gonzalez, T. A. Houser, J. A. Unruh, E. A. E. Boyle, and T. G. O'Quinn. 2016. The effect of branding on consumer palatability ratings of beef strip loin steaks. J. Anim. Sci. 94:4930–4942. https://doi.org/10.2527/jas2016-0893.

Supplemental Table 1. Effect of supranutritional zinc (Zn) and ractopamine hydrochloride (RAC) supplementation on quality characteristics of beef *longissimus thoracis* steaks at different postmortem timepoints.

	CON[1]		SUPZN[1]		P-value		
Item	NO[2]	RAC[2]	NO[2]	RAC[2]	ZNTRT	RACTRT	ZNTRT × RACTRT
Percent Purge[3]							
7 Days Postmortem	2.1 ± 0.2	2.3 ± 0.2	2.2 ± 0.2	2.3 ± 0.2	0.78	0.38	0.69
14 Days Postmortem	2.2 ± 0.2	2.3 ± 0.2	2.0 ± 0.2	1.9 ± 0.2	0.07	0.95	0.52
28 Days Postmortem	1.5 ± 0.1	1.7 ± 0.2	1.6 ± 0.1	1.5 ± 0.1	0.93	0.61	0.31
42 Days Postmortem	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	0.52	0.38	0.66
pH							
7 Days Postmortem	5.51 ± 0.02	5.49 ± 0.02	5.49 ± 0.02	5.48 ± 0.02	0.38	0.35	0.83
14 Days Postmortem	5.52 ± 0.01	5.52 ± 0.01	5.51 ± 0.01	5.50 ± 0.01	0.27	0.59	0.74
28 Days Postmortem	5.55 ± 0.01	5.54 ± 0.01	5.53 ± 0.01	5.51 ± 0.01	0.03	0.20	0.76
42 Days Postmortem	5.59 ± 0.01	5.54 ± 0.01	5.57 ± 0.01	5.54 ± 0.01	0.44	< 0.01	0.35
Marbling Score[4]							
7 Days Postmortem	560 ± 30	470 ± 30	510 ± 30	490 ± 30	0.66	0.07	0.28
14 Days Postmortem	540 ± 30	470 ± 30	520 ± 30	460 ± 30	0.60	0.04	1.00
28 Days Postmortem	560 ± 30	480 ± 30	500 ± 30	460 ± 30	0.31	0.06	0.51
42 Days Postmortem	600 ± 40	530 ± 40	540 ± 40	480 ± 40	0.15	0.12	0.85
Percent Cook Loss[5]							
7 Days Postmortem	23.4 ± 0.9	23.5 ± 1.0	23.7 ± 0.9	25.5 ± 0.9	0.24	0.31	0.34
14 Days Postmortem	24.5 ± 1.1	26.0 ± 1.2	22.6 ± 1.1	24.5 ± 1.1	0.14	0.14	0.86
28 Days Postmortem	24.3 ± 0.9	24.3 ± 1.0	22.6 ± 0.9	26.0 ± 0.9	0.95	0.07	0.07
42 Days Postmortem	26.0 ± 0.9	24.9 ± 1.0	24.8 ± 0.9	25.9 ± 0.9	0.88	0.97	0.24
*L** [6]							
7 Days Postmortem	42.67 ± 0.55	42.61 ± 0.60	42.22 ± 0.55	41.99 ± 0.55	0.35	0.79	0.88
14 Days Postmortem	43.56 ± 0.55	44.33 ± 0.60	44.10 ± 0.55	43.78 ± 0.55	1.00	0.69	0.35
28 Days Postmortem	45.39 ± 0.52	45.65 ± 0.56	44.64 ± 0.52	44.61 ± 0.52	0.10	0.84	0.79
42 Days Postmortem	45.42 ± 0.57	45.72 ± 0.6	44.60 ± 0.57	44.17 ± 0.57	0.05	0.91	0.53
*a** [6]							
7 Days Postmortem	19.89 ± 0.27	19.22 ± 0.29	20.18 ± 0.27	19.59 ± 0.27	0.23	0.03	0.89
14 Days Postmortem	20.07 ± 0.35	19.64 ± 0.38	20.17 ± 0.35	20.55 ± 0.35	0.17	0.94	0.26
28 Days Postmortem	19.98 ± 0.44	19.31 ± 0.47	20.58 ± 0.44	20.21 ± 0.44	0.11	0.25	0.74
42 Days Postmortem	21.72 ± 0.43	21.22 ± 0.46	21.64 ± 0.43	22.20 ± 0.43	0.31	0.94	0.24
*b** [6]							
7 Days Postmortem	17.90 ± 0.26	17.43 ± 0.28	18.10 ± 0.26	17.55 ± 0.26	0.55	0.07	0.87
14 Days Postmortem	18.53 ± 0.26	18.62 ± 0.28	18.83 ± 0.26	19.02 ± 0.26	0.20	0.61	0.86
28 Days Postmortem	19.07 ± 0.27	18.72 ± 0.29	19.24 ± 0.27	19.09 ± 0.27	0.33	0.36	0.72
42 Days Postmortem	20.33 ± 0.25	20.04 ± 0.27	20.04 ± 0.25	20.22 ± 0.25	0.82	0.83	0.37
Hue Angle[7]							
7 Days Postmortem	41.99 ± 0.42	42.22 ± 0.46	41.89 ± 0.42	41.83 ± 0.42	0.59	0.85	0.74
14 Days Postmortem	42.69 ± 0.42	43.51 ± 0.45	43.05 ± 0.42	42.78 ± 0.42	0.67	0.53	0.21
28 Days Postmortem	43.67 ± 0.44	44.11 ± 0.47	43.11 ± 0.44	43.42 ± 0.44	0.17	0.41	0.88
42 Days Postmortem	43.14 ± 0.42	43.38 ± 0.46	42.81 ± 0.42	42.34 ± 0.42	0.13	0.80	0.42
Chroma[8]							
7 Days Postmortem	26.76 ± 0.31	25.95 ± 0.34	27.11 ± 0.31	26.30 ± 0.31	0.28	0.02	0.99
14 Days Postmortem	27.32 ± 0.39	27.06 ± 0.42	27.61 ± 0.39	28.00 ± 0.39	0.14	0.86	0.42

Supplemental Table 1. (*Continued*)

	CON[1]		SUPZN[1]		P-value		
Item	NO[2]	RAC[2]	NO[2]	RAC[2]	ZNTRT	RACTRT	ZNTRT × RACTRT
28 Days Postmortem	27.62 ± 0.46	26.90 ± 0.50	28.18 ± 0.46	27.80 ± 0.46	0.13	0.26	0.72
42 Days Postmortem	29.75 ± 0.44	29.19 ± 0.48	29.50 ± 0.44	30.03 ± 0.44	0.52	0.98	0.24

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg dry matter); SUPZN = CON + 60 mg Zn/kg dry matter from $ZnSO_4$ + 60 mg Zn/kg dry matter from Zn-amino acid complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN). Fed for the entire 89 d trial.

[2]NO = no supplemental RAC; RAC = 300 mg RAC per head per d (Actogain45; Zoetis, Parsippany, NJ) starting 28 d before harvest.

[3]Percent steak purge = (weight of package with purge- weight of package without purge/steak weight) × 100.

[4]Marbling Scores: 400 = small; 500 = modest; 600 = moderate.

[5]Steaks were cooked to an internal temperature of 68°C on clamshell grills. Percent cook loss = [(raw steak weight – cooked steak weight)/raw steak weight] × 100.

[6]Commission Internationale de l'Eclairage L^*, a^*, and b^* values were determined with a HunterLab Miniscan EZ instrument using illuminant D65 light source, 2.4 cm aperture, and a 10° observer angle.

[7]Hue angle was calculated using the following equation: arctangent (b^*/a^*).

[8]Chroma value was calculated using the following equation: $(a^{*2}+b^{*2})^{1/2}$.

CON-NO= No Zn or RAC supplementation. **CON-RAC=** No Zn supplementation, only RAC supplantation. **SUPZN-NO=** Zn supplementation, no RAC supplementation. **SUPZN-RAC=** Zn and RAC supplementation.