Meat and Muscle Biology™

The Influence of Supranutritional Zinc and Ractopamine Hydrochloride Supplementation on Early Postmortem pH Decline and Meat Quality Development of Beef

Matthew D. Schulte[1], Katherine G. Hochmuth[1], Edward M. Steadham[1], Kenneth J. Prusa[2], Steven M. Lonergan[1], Stephanie L. Hansen[1], and Elisabeth Huff-Lonergan[1]*

[1]Department of Animal Science, Iowa State University, Ames, Iowa 50011, USA
[2]Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa 50011, USA
*Corresponding author. Email: elonerga@iastate.edu (Elisabeth Huff-Lonergan)

Abstract: The objectives of this experiment were to determine the impact of zinc (Zn) and ractopamine hydrochloride (RH) supplementation of beef steers on early postmortem pH decline and meat quality development of aged *longissimus thoracis* steaks. Angus steers ($n = 20$; 516 kg average initial body weight) were fed in a 2 × 2 factorial and equally assigned to Zn and RH treatments: control (analyzed 36 mg Zn/kg dry matter [DM]) or supranutritional Zn supplementation (control diet + 60 mg Zn/kg DM [from $ZnSO_4$] + 60 mg Zn/kg DM [from Zn–amino acid complex]) dietary treatments for the entire 89-d trial. Starting 28 d before harvest, steers were blocked by body weight within Zn treatments to RH treatments of 0 or 300 mg per steer per day. Steers were harvested, and the following data were collected: *longissimus thoracis* pH and temperature (1, 3, 6, and 24 h postmortem), carcass measurements, and quality attributes of aged steaks (1, 3, 7, or 14 d postmortem). Muscle samples were taken at 1 h and 1, 3, 7, and 14 d postmortem for biochemical analysis. Supplementation of supranutritional Zn trended for a lower (pH 5.49; $P = 0.06$) pH in samples at 6 h postmortem and lower (5.40 kg; $P = 0.06$) Warner-Bratzler shear force value at 1 d postmortem. Supplementation of RH in samples resulted in a higher (pH 5.86; $P = 0.04$) pH at 6 h postmortem, greater (7.64 kg; $P < 0.01$) Warner-Bratzler shear force value at 1 d postmortem, and lesser whole muscle desmin ($P = 0.05$) and troponin-T ($P = 0.04$) degradation at 1 d postmortem. The observed differences in this study lends further credence to the need to more fully understand the role of Zn and RH in muscle growth and early postmortem metabolism because of potential impacts on beef quality.

Key words: beef quality, pH decline, proteolysis, ractopamine hydrochloride, tenderness, zinc
Meat and Muscle Biology 5(1): 37, 1–15 (2021) **doi:10.22175/mmb.12250**
Submitted 28 January 2021 Accepted 25 May 2021

Introduction

Tenderness is a primary trait that affects consumer satisfaction of beef (Wilfong et al., 2016). Still, efficient growth of beef cattle must also be considered because it is key to the sustainability of beef cattle production. Optimizing efficient growth by using nutritional management practices, such as feeding zinc (Zn) above the National Academy of Sciences, Engineering, and Medicine (2016) recommendations (i.e., supranutritional; Zn in the basal diet + 120 mg Zn/kg dry matter [DM] supplemented [Genther-Schroeder et al., 2018]), and growth-promoting technologies, such as the beta agonist ractopamine hydrochloride (RH), have the potential to positively impact the efficiency of beef cattle growth (Genther-Schroeder et al., 2016a, 2016b; Ebarb et al., 2017; Reinhardt et al., 2019). The impact of Zn supplementation can vary depending on source and concentration as some literature has noted no differences in cattle growth when cattle were supplemented 160 mg Zn/kg on a DM basis (Bohrer et al., 2014). Zinc is an essential trace mineral that is critical for

many biological processes, including muscle growth and metabolism (Park et al., 1986), and may work synergistically with RH to optimize the growth of beef cattle (Genther-Schroeder et al., 2016b; Reinhardt et al., 2019).

Tenderness and the factors affecting tenderness development are among the most studied facets of meat science. Beef tenderness development is impacted by a variety of factors, including rate and extent of pH decline (Marsh et al., 1987; Wu et al., 2014), sarcomere length (England et al., 2012), postmortem protein degradation (Huff-Lonergan et al., 1996; England et al., 2012), and collagen content (Nishimura et al., 2009). However, the impact of feeding supranutritional Zn and RH synergistically on beef tenderness and quality development is not fully understood. Bohrer et al. (2014) identified no difference in shear force values (14 d postmortem) of steaks from cattle supplemented with RH only and with RH with 160 mg Zn/kg and 1.25 mg chromium/kg on a DM basis (Bohrer et al., 2014). Previous investigations into the antemortem sarcoplasmic muscle proteome of cattle supplemented with supranutritional Zn and RH fed in combination influenced the abundance of proteins essential to carbohydrate metabolism (Genther-Schroeder et al., 2016c). Differences in carbohydrate metabolism have been identified as potential sources of variability in beef tenderness development (Antonelo et al., 2020). Zinc is known to impact phosphorylation of proteins (Haase and Maret, 2003; Lee et al., 2009), thus having the capacity to alter proteins/enzymes involved in metabolism. This could ultimately influence pH decline in meat products through potential modulation of phosphorylation of proteins essential to metabolism. Therefore, it was hypothesized that RH and Zn supplementation would influence meat quality by affecting early postmortem pH decline, thus influencing tenderness development. The objective of this experiment was to determine the impact of supplementation of supranutritional Zn and RH of finishing beef steers on early postmortem pH decline and meat quality development of aged *longissimus thoracis* (LT) steaks.

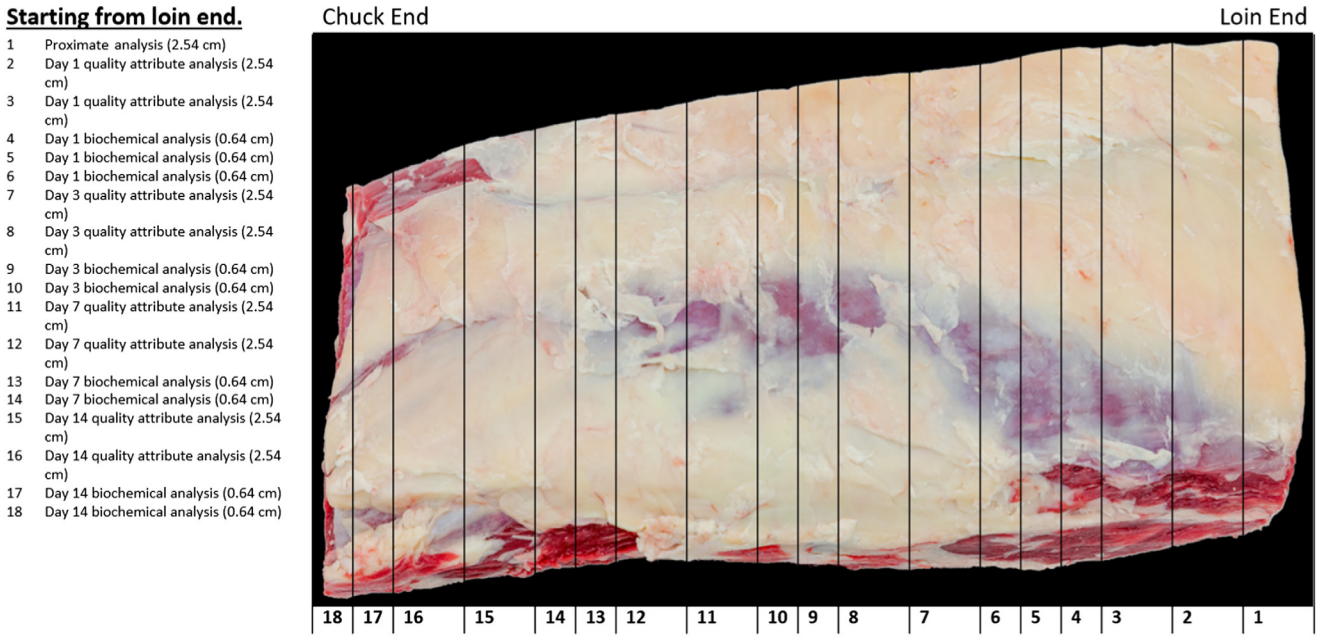
Materials and Methods

Live animal procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (#11-17-8645-B). Twenty high-percentage Angus steers (~516 kg initial body weight) were obtained from a sole source and fed one of 4 diets using an individual feed intake monitoring system (GrowSafe bunks; GrowSafe Systems Ltd., Airdrie, Alberta, Canada). All steers were fed the same basal diet. Steers were assigned to Zn and RH treatments based on similar growth potential (GeneMax gain score; GeneMax Focus, Zoetis, Parsippany, NJ) and initial body weight. Zinc supplementation occurred for the entire 89-d trial and consisted of the following treatments: a non-Zn supplemented control (CON; analyzed 36 mg Zn/kg DM) or supranutritional Zn (SUPZN; CON + 60 mg Zn/kg DM [as $ZnSO_4$] + 60 mg Zn/kg DM [as Zn-amino acid complex; Availa-Zn; Zinpro, Eden Prairie, MN]). Starting 28 d before harvest, RH treatments consisted of 0 (NO) or 300 mg of RH per steer per day (RAC; Actogain45, Zoetis, Parsippany, NJ). At finishing weights (~749 kg), steers were weighed at the Iowa State University Beef Nutrition Research Farm and transported to the Iowa State University Meat Laboratory, where they were held in lairage for approximately 17 h. One steer per treatment ($n = 4$) was harvested under USDA inspection at the Iowa State University Meat Laboratory on 5 different dates ($N = 20$). The total harvest day range was across 26 d in a single month (November). Harvest was completed using standard protocols in the United States, and carcasses were not electrically stimulated. Stunning was completed using a captive-bolt pistol prior to exsanguination. *Longissimus* muscle temperature and pH were measured between the 12th and 13th rib of the right side of the carcass at 1, 3, 6, and 24 h post exsanguination using a Hanna HI9025 pH meter (Hanna Instruments, Woonsocket, RI). Each subsequent postmortem muscle temperature and pH measurement wase taken 2.54 cm anterior of the previous measurement. Calibration of the pH meter and accuracy of measurements were verified using pH 4 and 7 buffers at harvest floor room temperatures (22°C). Accuracy was monitored using the pH 7 buffer and was recalibrated if pH readings in this buffer fell out of range (pH range 6.95 to 7.05). Accuracy of measurement was determined between each sample measurement. A muscle sample (150 g) was excised between the 12th and 13th rib on the right side of the carcass at 1 h postmortem after 1-h pH and temperature measurements were obtained. This sample was immediately cut into small cubes and stored at −80°C for protein analysis. Two trained individuals collected carcass yield (dressing percentage; ribeye area [REA]; fat thickness; kidney, pelvic, and heart fat percentage [KPH]; and yield grade [YG]) and quality (marbling score; Small = 400, Modest = 500) data. Dressing percentage of each carcass was calculated using the following equation: hot carcass weight (HCW)/live weight × 100. Yield grade

values were calculated using the following equation: 2.5 + (2.5 × fat thickness) + (0.0038 × HCW) + (0.2 × KPH) − (0.32 × REA). Rib sections from the carcasses' left sides were fabricated at 24 h postmortem into nine 2.54-cm-thick boneless steaks for quality attribute analysis (Figure 1). An additional nine 0.64-cm-thick steaks containing only the LT muscle were fabricated for biochemical analysis (Figure 1). All steaks were vacuum packaged, aged (2°C) for 1, 3, 7, or 14 d, and frozen until quality (frozen at −29°C) or biochemical analysis (frozen at −80°C; Figure 1). Steaks from each postmortem timepoint were thawed (24 h at 2°C), and purge, pH, Hunter L, a, and b values, and marbling score data were collected on each pair of steaks. Purge was collected by weighing the steak and the package with the purge remaining in the package and then removing the steak to obtain the weight of the package plus the purge. Purge was calculated using the following formula: [(weight of the package with purge − weight of the package without purge)/(steak weight in the package with purge – the weight of the package without purge)] × 100. Steaks were removed from packages and allowed to bloom for 30 min at room temperature (~22°C) before color analysis. Surface color measurements (L, a, and b values) were collected using a Commission Internationale de l'Eclairage (CIE) L^*, a^*, and b^* color space (L^* = lightness; a^* = redness; b^* = yellowness). Surface color measurements were taken using a HunterLab MiniScan EZ 4500L colorimeter (Hunter Associates Laboratory Inc., Reston, VA) at a 10° observer angle, using illuminant D65 (daylight at 6,500 K), and a 2.4-cm aperture size. Hue angle and chroma values were calculated using the following formulas: hue angle = arctangent (b^*/a^*) and chroma = $(a^{*2} + b^{*2})^{1/2}$ (American Meat Science Association, 2012). pH values were collected on each steak as described earlier. Steaks were cooked on clamshell grills (Cuisinart, East Windsor, NJ) to an internal temperature of 68°C, and cook loss was determined using the following formula: [(raw steak weight − cooked steak weight)/raw steak weight] × 100. A minimum of three 1.27-cm cores per steak were removed parallel to the muscle fibers for Warner-Bratzler shear force (WBSF) analysis (American Meat Science Association, 2016). This resulted in a total of at least 6 cores per postmortem aging timepoint per animal. A WBSF attachment (100 kg compression load cell and crosshead speed of 3.3 mm/s) was fitted to an Instron (Instron, Norwood, MA) for analysis.

Proximate composition

A food processor (KitchenAid, St. Joseph, MI) was used to finely mince raw steak (~200 g) containing only the LT muscle from the 12th rib to analyze protein, fat, and moisture content in duplicate. Total fat and moisture content were determined using AOAC methods (AOAC International, 2013). Fat was evaluated with the CEM ORACLE System (AOAC International, 2013; CEM Corporation Matthews,



NC), and moisture was evaluated with the CEM SMART 6 System (AOAC International, 2013; CEM Corporation). Protein content was measured with the CEM Sprint Rapid Protein Analyzer (AOAC International, 2011; CEM Corporation).

Whole muscle protein extraction

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

Sarcoplasmic protein extraction

Frozen meat samples containing only the LT (200 g) were homogenized and powdered in liquid nitrogen. Samples from each postmortem timepoint (3 g) were homogenized, and sarcoplasmic proteins were extracted (4°C; 50 mM Tris-HCl and 1 mM ethylenediaminetetraacetic acid [pH 8.0]) as described by Carlson et al. (2017a).

Gel electrophoresis running conditions

The samples from the whole muscle preparation were used to evaluate degradation of desmin and troponin-T (1 h and 1, 3, 7, and 14 d postmortem) and calpain-1 autolysis (1 h and 1, 3, and 7 d postmortem) using one-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) as described by Carlson et al. (2017a, 2017b). Desmin degradation products that were soluble in the sarcoplasmic fraction (1 h and 1, 3, 7, and 14 d postmortem) and sarcoplasmic calpain-1 autolysis (1 h and 1, 3, and 7 d postmortem) were determined using one-dimensional SDS-PAGE as described by Carlson et al. (2017a, 2017b). A reference sample, composed of 4 samples at 1 d postmortem and 4 samples from the same animals at 7 d postmortem, was created. This day 1/day 7 mixed reference sample was loaded on each gel for all western blot analysis. SE 260 Hoefer Mighty Small II electrophoresis units (Hoefer, Inc., Holliston MA) were used to run 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] tetramethylenediamine, 0.05% [wt/vol] ammonium persulfate, 0.5 M Tris-HCl [pH 8.8]) for desmin and troponin-T analysis and 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] tetramethylenediamine, 0.05% [wt/vol] ammonium persulfate, 0.5 M Tris-HCl [pH 8.8]) for calpain-1 autolysis. Protein samples (40 µg) were loaded into each well for whole muscle extract desmin and troponin-T analysis along with whole muscle extract and soluble sarcoplasmic calpain-1 autolysis. For analysis of soluble desmin degradation products in the sarcoplasmic fraction, 50 µg of protein was loaded into each well.

Transferring conditions and western blot analysis

After running, the SDS-PAGE gels were transferred to polyvinylidene difluoride membranes with pore sizes of 0.2 µm (Immobilon-PSQ, 26.5 by 3.75 M RL, VCAT#ISEQ00010, Millipore Corporation, Billerica, MA) as described by Carlson et al. (2017b). Western blot analysis was conducted as described by Carlson et al. (2017a, 2017b). The following primary antibody concentrations were made by diluting with phosphate-buffered saline (PBS)-Tween and added to separate blots: desmin (1:40,000) using polyclonal rabbit anti-desmin antibody produced at Iowa State University (Huff-Lonergan et al., 1996; Carlson et al., 2017a, 2017b), troponin-T (1:10,000) using monoclonal mouse anti-troponin-T primary antibody (T6277, JLT-12; Sigma-Aldrich, St. Louis, MO), and calpain-1 (1:5,000) using monoclonal mouse anti-calpain-1 (MA3-940; Thermo-Scientific, Rockford, IL). All blots were incubated for 1 h at room temperature (~22°C) with primary antibodies. Desmin and troponin-T secondary antibodies were diluted with PBS-Tween, and calpain-1 secondary antibodies were incubated in PBS-Tween mixed with 5% nonfat dry milk. Secondary antibodies were added to separate blots following washes at the following concentrations: desmin (1:20,000): goat anti-rabbit horseradish peroxidase (HRP) antibody (32430; Thermo-Scientific); troponin-T (1:5,000): goat anti-mouse HRP antibody (32430; Pierce); and calpain-1 (1:10,000): goat anti-mouse HRP antibody (A2554; Sigma Aldrich, St. Louis, MO). All blots were incubated for 1 h at room temperature (~22°C) with secondary antibodies. Following incubation with secondary antibodies, desmin and troponin-T blots were washed with PBS-Tween 3 times for 10 min, and calpain-1 blots were washed 5 times for 10 min. Blots were analyzed as described by Carlson et al. (2017a, 2017b). Using the internal reference on each blot, the intensities of the 38-kDa desmin degradation product and a 30-kDa troponin-T degradation product were quantified as a comparative ratio of the sample protein band to the internal

reference protein band on 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.

Statistics

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 RH and the interaction. Harvest group was included as a fixed effect for all analyses. Whole muscle extract desmin and troponin-T degradation products, soluble sarcoplasmic desmin degradation products, and whole muscle extract and soluble sarcoplasmic calpain-1 data were analyzed using the Mixed procedure of SAS (version 9.4; SAS Institute) as a 2×2 factorial with fixed effects of Zn and RH and the interaction. Gel was used as a random effect in the model. Pearson correlations were generated using PROC CORR using the following criteria: lowly correlated ($r \leq 0.35$), moderately correlated ($0.36 \leq r \leq 0.67$), and highly correlated ($r \geq 0.68$; Taylor, 1990). Normality was confirmed using the Shapiro-Wilk test. Cook's D was used to determine outliers at Cook's D ≥ 0.5. Significance levels for all analyses were set at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. Least-squares means and standard errors were reported for all measured attributes. Least-squares means were separated using SAS's PDIFF procedure (version 9.4; SAS Institute Inc., Cary, NC).

Results and Discussion

Postmortem pH decline and Warner-Bratzler shear force values

The rate of pH decline and muscle temperature have been shown to interact and impact ultimate pH and tenderness development in beef (Hwang and Thompson, 2001a; Hopkins et al., 2014; Warner et al., 2014). This very complex biological reaction is still not fully defined. Early studies of this subject demonstrated differences in the rate of pH decline of different beef muscles even when the temperature was kept at a constant state (Bendall, 1978). This variation in rate of pH decline was originally thought to simply be a mechanism of excess calcium and adenosine triphosphate turnover in the muscle system but is now deemed far more complex. Several studies have demonstrated that an intermediate rate of pH decline results in the most tender beef products after aging (Marsh et al., 1987; Hwang and Thompson, 2001a). It has also been demonstrated that a rapid rate of pH decline early postmortem, typically due to electrical stimulation, can result in more tender beef products at 1 d postmortem (Hwang and Thompson, 2001b). These studies further demonstrate the complex relationship between early postmortem pH decline and tenderness development. In this study, postmortem pH, temperature, and WBSF values were not affected by the interaction of Zn and RH, so the main effects will be discussed for the remainder of this section.

A trend (Figure 2; $P = 0.06$) for lower pH at 6 h postmortem was observed from steers supplemented with supranutritional Zn, specifically in the Zn-only (SUPZN-NO [pH 5.49]) treatment. Conversely, a higher (Figure 2; $P = 0.04$) pH was observed in muscle from steers supplemented with RH compared with steers not supplemented with RH, specifically in CON-RAC treatment (pH 5.86), at 6 h postmortem. These 2 effects offset one another in the combination treatment—SUPZN-RAC (pH 5.70)—showing greater similarity to CON-NO (pH 5.68). These effects were not influenced by differences in muscle temperature at any timepoint (Figure 3). This difference in the rate of pH decline followed a similar trend in the observed differences in WBSF values at 1 d postmortem. Supplementation of RH resulted in greater (Figure 4; $P < 0.01$) WBSF values at 1 d postmortem, whereas supranutritional Zn supplementation trended (Figure 4; $P = 0.06$) for a lower WBSF value at 1 d postmortem. Specifically, supplementation of supranutritional Zn alone (SUPZN-NO; 5.40 kg) resulted in a 2.2-kg-lower WBSF value at 1 d postmortem than supplementation of RH alone (CON-RAC; 7.64 kg). Compared with the control treatment (6.72 kg; CON-NO), SUPZN-NO demonstrated a 1.3-kg-lesser WBSF value at 1 d postmortem. Supplementation of RH has consistently resulted in greater WBSF values at 3–14 d postmortem in the *longissimus lumborum* of beef compared with cattle not supplemented with RH (Avendaño-Reyes et al., 2006; Scramlin et al., 2010; Boler et al., 2012; Bohrer et al., 2014; Lean et al., 2014). The consistency of greater WBSF values in steaks from cattle supplemented with RH remains unless steaks are aged for extended periods (≥14 d; Scramlin et al., 2010; Boler et al., 2012; Bohrer et al., 2014). This delay in onset of or slower rate of tenderization could provide the opportunity to investigate and understand biological factors that influence a delayed or hastened rate of tenderization, even if tenderness differences do not persist later postmortem (≥14 d). An understanding of this

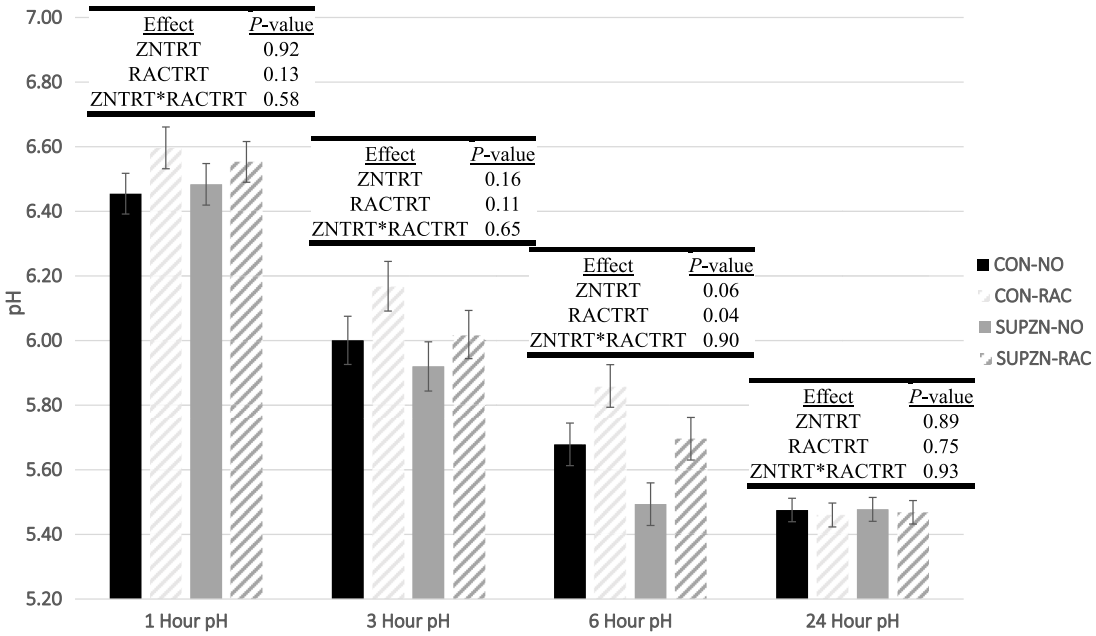


Figure 2. Effect of supranutritional zinc (Zn) and ractopamine hydrochloride (RH) supplementation on pH values of beef *longissimus thoracis* muscle at different postmortem timepoints.[1,2,3]

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg dry matter [DM]); SUPZN = CON + 60 mg Zn/kg DM (from ZnSO4) + 60 mg Zn/kg DM (from Zn–amino acid complex; Availa-Zn; Zinpro Corporation, Eden Prairie, MN). Fed for the entire 89-d trial.

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

[3]Means and standard errors of the mean are reported.

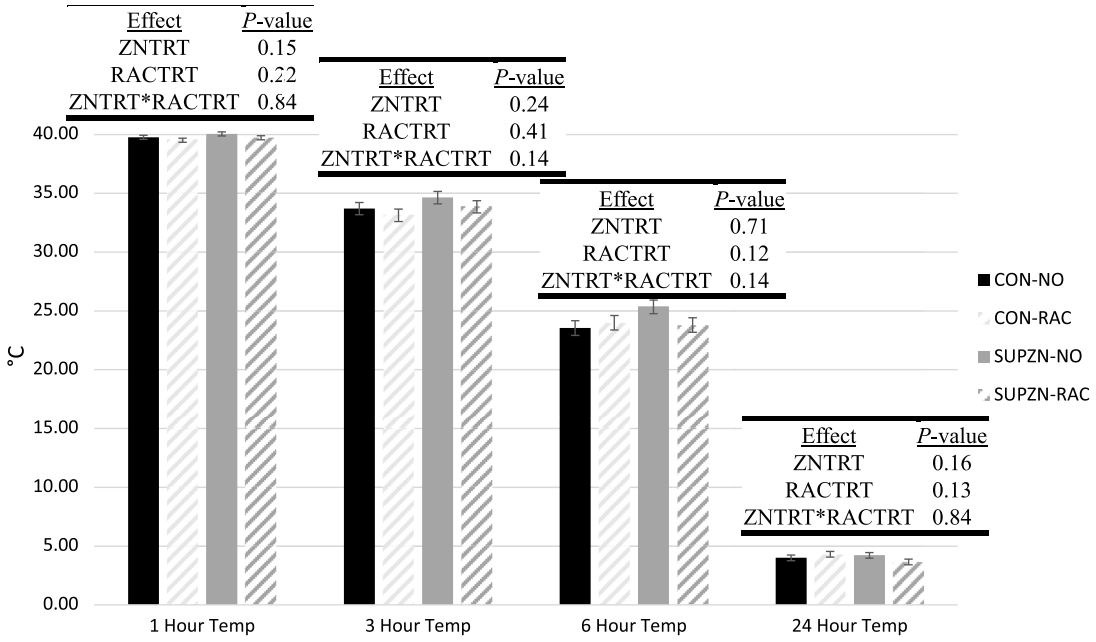


Figure 3. Effect of supranutritional zinc (Zn) and ractopamine hydrochloride (RH) supplementation on temperature values of beef *longissimus thoracis* muscle at different postmortem timepoints.[1,2,3]

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg dry matter [DM]); SUPZN = CON + 60 mg Zn/kg DM (from ZnSO4) + 60 mg Zn/kg DM (from Zn–amino acid complex; Availa-Zn; Zinpro Corporation, Eden Prairie, MN). Fed for the entire 89-d trial.

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

[3]Means and standard errors of the mean are reported.

biological difference in rate of tenderness development could provide for application of alternative merchandizing strategies of beef products.

The differences between the treatments in pH decline at 6 h postmortem and WBSF values at 1 d postmortem are strikingly similar in the observed figures

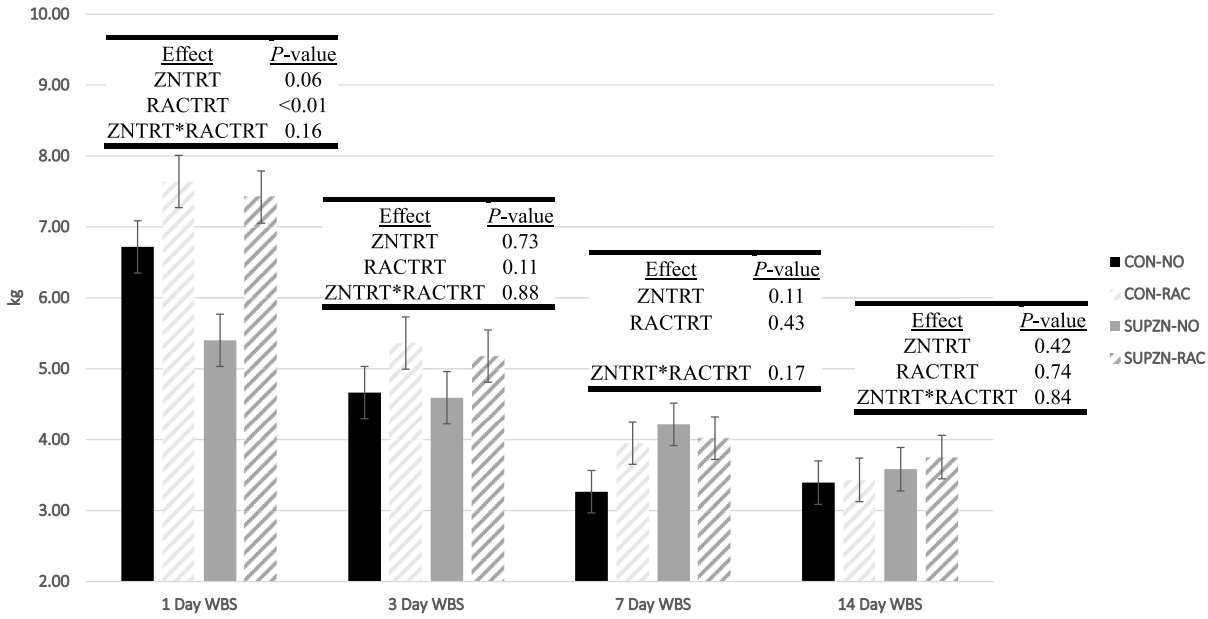


Figure 4. Effect of supranutritional zinc (Zn) and ractopamine hydrochloride (RH) supplementation on Warner-Bratzler shear force (WBS) values of beef *longissimus thoracis* steaks at different postmortem timepoints.[1,2,3,4]

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg dry matter [DM]); SUPZN = CON + 60 mg Zn/kg DM (from ZnSO4) + 60 mg Zn/kg DM (from Zn–amino acid complex; Availa-Zn; Zinpro Corporation, Eden Prairie, MN). Fed for the entire 89-d trial.

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

[3]WBS values were averaged across 2 adjacent steaks.

[4]Means and standard errors of the mean are reported.

(Figures 2 and 4). Correlation coefficients found in Table 1 demonstrate a moderately strong positive correlation of pH values at 3 h ($r = 0.50$; $P = 0.03$) and 6 h ($r = 0.55$; $P = 0.01$) postmortem with WBSF values at 1 d postmortem. This relationship is consistent with Hwang and Thompson (2001a) observations, demonstrating a lower early postmortem WBSF value in beef muscles with the most rapid rate of pH decline. These data further underscore the need to investigate the biochemistry underlying the relationship of early postmortem pH decline and metabolism with tenderness development.

Consistent evidence of the relationship of pH to tenderness supports the hypothesis that pH has an influence on biochemical factors that influence tenderness. Wu et al. (2014) classified *longissimus dorsi* muscle from bull carcasses into high (≥6.2), intermediate (5.80–6.19), and low (≤5.79) ultimate pH classifications based on 48-h pH measurements. Beef with highest ultimate pH resulted in the lowest WBSF value

Table 1. Pearson correlations between postmortem pH, temperature (Temp), and Warner-Bratzler shear force (WBSF) values[1]

	pH 3 h	pH 6 h	pH 24 h	Temp 1 h	Temp 3 h	Temp 6 h	Temp 24 h	WBSF 1 d	WBSF 3 d	WBSF 7 d	WBSF 14 d
pH 1 h	**0.56**	*0.41*	0.2	−0.21	0.03	0.05	0.11	0.11	0.11	0.29	−0.04
pH 3 h		**0.89**	−0.03	−0.34	−0.11	−0.07	−0.21	**0.50**	0.02	−0.28	−0.17
pH 6 h			−0.03	*−0.40*	−0.15	−0.16	−0.34	**0.55**	−0.03	**−0.44**	−0.33
pH 24 h				0.17	−0.01	−0.01	−0.17	−0.03	0.33	0.19	*0.39*
Temp 1 h					−0.09	−0.03	0.01	−0.22	0.34	0.34	*0.39*
Temp 3 h						**0.66**	**0.55**	**−0.50**	**−0.55**	−0.15	*−0.38*
Temp 6 h							**0.67**	*−0.42*	**−0.52**	0.06	−0.27
Temp 24 h								**−0.63**	**−0.53**	0.17	−0.25
WBSF *1* d									**0.58**	−0.16	0.15
WBSF 3 d										**0.44**	**0.67**
WBSF 7 d											**0.57**

[1]Significant correlations are bolded ($P \leq 0.05$). Trending correlations are italicized ($0.05 < P \leq 0.10$).

at each day of aging (1, 2, 7, 14, 21, and 28 d postmortem) (Wu et al., 2014). The intermediate ultimate pH classification resulted in the greatest WBSF values across all postmortem aging timepoints, and the lowest ultimate pH classification was in between the 2 classifications (Wu et al., 2014). In the current study, a trend for a moderately strong positive correlation was determined between the pH at 24-h-postmortem and 14-d-postmortem WBSF values ($r = 0.39$; $P = 0.09$). The 24-h pH values ranged from 5.38 to 5.62 in this study; thus, we did not have the extremes of ultimate pH values that Wu et al. (2014) suggested could strongly influence product quality.

As stated earlier, an intermediate rate of pH decline results in the most tender beef products (Marsh et al., 1987; Hwang and Thompson, 2001b). A moderately strong negative correlation was observed between 6-h pH and 7-d WBSF values ($r = -0.44$; $P = 0.05$), demonstrating a less extreme rate of pH decline resulting in a more tender product when given proper aging time (≥7 d).

The rate of pH and temperature decline are continuously interacting and influencing each other, thus collaborating to impact tenderness development (Hopkins et al., 2014; Warner et al., 2014). In this study, no differences in muscle temperature during harvest were observed among treatments or their interaction at any postmortem timepoint (Figure 3). A trend for a moderately strong negative correlation of pH at 6 h postmortem with temperature at 1 h postmortem was determined (Table 1; $r = -0.40$; $P = 0.08$). This correlation supports the hypothesis that a higher initial temperature can increase the rate of postmortem glycolysis, thus causing a more rapid pH decline (Kim et al., 2014). A moderately strong negative correlation was observed between temperature at 3 h ($r = -0.50$; $P = 0.02$), 6 h ($r = -0.42$; $P = 0.06$), and 24 h ($r = -0.63$; $P < 0.01$) postmortem and WBSF values at 1 d postmortem. Similarly, temperature at 3 h ($r = -0.55$; $P = 0.01$), 6 h ($r = -0.52$; $P = 0.02$), and 24 h ($r = -0.53$; $P = 0.02$) postmortem showed a moderately strong negative correlation with WBSF values at 3 d postmortem. This relationship between prerigor temperatures and early postmortem (1–3 d) tenderness development has been previously demonstrated (Hwang et al., 2004; Kim et al., 2014). Hwang et al. (2004) showed a lower WBSF value at 24 h postmortem in beef *longissimus dorsi* muscle that was held at a higher prerigor temperature after harvest. This difference in WBSF value was attributed to postmortem proteolysis variations. These correlations from this small data set are consistent with previous reports of the strong relationship between postmortem pH decline, temperature decline, and tenderness development in beef steaks from the LT.

Carcass characteristics and proximate analysis

The use of RH to improve growth performance and carcass lean yield has been extensively studied (Boler et al., 2012; Bohrer et al., 2014; Bittner et al., 2016, 2017; Genther-Schroeder et al., 2016b; Hagenmaier et al., 2017). However, many results related to these characteristics are inconsistent. Live weight, HCW, dressing percentage, and REA typically improve due to RH supplementation (Avendaño-Reyes et al., 2006; Boler et al., 2012; Bohrer et al., 2014). However, in this study, carcass characteristics were not affected by supplementation of Zn, RH, or the interaction (Table 2; $P \geq 0.17$). Several previous studies have also demonstrated no difference in these lean yield characteristics of carcasses from cattle supplemented with RH (Avendaño-Reyes et al., 2006; Samuelson et al., 2016; Trotta et al., 2019).

No difference in 12th rib backfat, KPH, YG, or marbling score was observed in this study due to supplementation of Zn, RH, or their interaction (Table 2; $P \geq 0.17$). Several studies have observed lesser fat thickness and KPH along with lower marbling scores and YG in carcasses from cattle supplemented with RH (Bohrer et al., 2014; Bittner et al., 2016; Trotta et al., 2019). Conversely, studies have shown no difference in carcass characteristics from cattle supplemented with RH (Samuelson et al., 2016; Trotta et al., 2019). These inconsistencies are thought to be due to several factors, including diet, background, dosage, length of time of dosage, the power of the study, and genetic variations.

Proximate results determined no difference in protein, moisture, or fat percentages (Table 2; $P > 0.10$) due to supplementation of Zn, RH, or their interaction.

Limited differences in quality characteristics existed. Data for these characteristics can be found in Supporting Information Table S1.

Calpain-1 autolysis and desmin and troponin-T postmortem protein degradation

The calpain proteolytic system, specifically calpain-1, is the primary contributor to postmortem protein degradation of myofibrillar, cytoskeletal, and intermediate filament proteins in postmortem muscle (Geesink et al., 2006; Koohmaraie and Geesink,

Table 2. Effect of supranutritional zinc (Zn) and ractopamine hydrochloride (RH) supplementation on carcass characteristics and proximate composition of beef finishing steers *longissimus thoracis* muscle

	CON[1]		SUPZN[1]		SEM				*P* Value		
Item	NO[2]	RAC[2]	NO[2]	RAC[2]	CON-NO	CON-RAC	SUPZN-NO	SUPZN-RAC	ZN TRT	RAC TRT	ZN TRT × RAC TRT
Steers (*n*)	5	5	5	5							
Initial BW, kg	512	516	519	516	4.0	4.0	4.0	4.0	0.35	0.84	0.36
Live Weight, kg	712	715	712	700	9.8	9.3	9.6	9.3	0.44	0.64	0.46
Hot Carcass Weight, kg	436	437	438	443	3.2	3.3	3.2	3.3	0.22	0.39	0.57
Dress, %	61.4	61.6	61.8	62.4	0.5	0.5	0.5	0.5	0.22	0.41	0.58
Ribeye Area, cm^2	87.4	91.7	87.8	86.7	2.4	2.4	2.4	2.4	0.35	0.52	0.28
12th Fat Thickness, cm	1.41	1.23	1.52	1.45	0.19	0.19	0.19	0.19	0.41	0.51	0.77
KPH, %	1.7	1.6	1.7	1.5	0.1	0.1	0.1	0.1	0.68	0.23	0.36
Yield Grade[3]	3.6	3.1	3.7	3.6	0.3	0.3	0.3	0.3	0.29	0.37	0.47
Marbling Score[4]	460	480	500	429	40	40	40	40	0.85	0.50	0.17
Protein, %[5]	22.3	22.7	22.3	22.9	0.3	0.3	0.3	0.3	0.71	0.12	0.69
Fat, %[5]	5.4	5.9	6.5	5.0	0.8	0.8	0.8	0.8	0.93	0.56	0.23
Moisture, %[5]	71.7	71.4	71.1	71.8	0.5	0.5	0.5	0.5	0.80	0.68	0.35

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg dry matter [DM]); SUPZN = CON + 60 mg Zn/kg DM (from ZnSO4) + 60 mg Zn/kg DM (from Zn–amino acid complex) (Availa-Zn; Zinpro Corporation, Eden Prairie, MN). Fed for the entire 89-d trial.

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

[3]Yield grade was calculated using the following equation: 2.5 + (2.5 * Fat Thickness) + (0.0038 * HCW) + (0.2 * KPH) − (0.32 * REA).

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

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

Measurements were taken from the left side of the carcass between the 12th and 13th rib at ~24 h postmortem.

BW = body weight; HCW = hot carcass weight; KPH = kidney, pelvic, and heart fat percentage; REA = ribeye area; SEM = standard error of the mean; TRT = treatment.

2006). Autolysis of calpain-1 (76-kDa product) negatively correlates with intact desmin, vinculin, and talin in pork (Bee et al., 2007). A variety of factors can influence calpain-1 activity, including pH decline (Melody et al., 2004), oxidative conditions (Rowe et al., 2004; Maddock Carlin et al., 2006), nitric oxide (Li et al., 2014; Liu et al., 2016, 2019; Zhang et al., 2018; Hou et al., 2020), and calpastatin activity (Pringle et al., 1993; Rowe et al., 2004). Zinc infused at high levels via the vascular system in carcasses immediately postmortem has been shown to limit calpain activity (Koohmaraie, 1992). Investigation into different fractions of muscle, such as the sarcoplasmic and myofibrillar fractions, has demonstrated that myofibrillar bound calpain-1 is nearly inactive proteolytically and arises from precipitation of calpain-1 onto the myofibril (Boehm et al., 1998). Calpain-1 autolysis in the whole muscle and in the sarcoplasmic fraction was not impacted by supplementation of Zn, RH, or their interaction (Table 3; $P \geq 0.06$). The observed trends for greater 78-kDa and lesser 76-kDa calpain-1 (Table 3; $P < 0.10$) in the sarcoplasmic fraction of muscle from cattle supplemented with supranutritional Zn is interesting. In ovine muscle, Koohmaraie (1992) infused high levels of Zn chloride (1 mM) to examine the impact of Zn infusion on postmortem proteolysis and observed reduced calpain-1 activity (2.4% of maximal activity) in the Zn-treated muscle. In the study by Koohmaraie (1992), it was concluded that Zn reduced the rate of calpain-1 autolysis rather than completely inhibiting autolysis of calpain-1. This relationship between calpain-1 autolysis and Zn needs to be studied further under physiological conditions. Additionally, a faster rate of pH decline has been demonstrated to be related to a more rapid rate of calpain-1 autolysis in the *psoas major* of pork (Melody et al., 2004). Differences in the rate of pH decline could be impacting differences in calpain-1 activity and subsequent loss of activity earlier postmortem. Although activity was not measured in this study, several studies have demonstrated the relationship between autolysis and activity of calpain-1 in postmortem muscle (Boehm et al. 1998; Melody et al., 2004).

Postmortem protein degradation significantly impacts tenderness development (Wu et al., 2014). Desmin and troponin-T degradation have commonly

Table 3. Effect of supranutritional zinc (Zn) and ractopamine hydrochloride (RH) supplementation on whole-muscle extract and soluble sarcoplasmic calpain-1 autolysis of beef *longissimus thoracis* muscle at different postmortem timepoints

		CON[1]		SUPZN[1]		SEM				*P* Value		
Item		NO[2]	RAC[2]	NO[2]	RAC[2]	CON-NO	CON-RAC	SUPZN-NO	SUPZN-RAC	ZN TRT	RAC TRT	ZN TRT × RAC TRT
Calpain-1, WM[3]												
1 Hour	80 kDa[4]	100.0	100.0	100.0	100.0	ND	ND	ND	ND	ND	ND	ND
	78 kDa[4]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	76 kDa[4]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1 Day	80 kDa[4]	38.2	42.0	35.8	42.2	7.7	7.7	7.7	7.7	0.89	0.52	0.87
	78 kDa[4]	32.6	33.2	33.4	32.4	2.1	2.1	2.1	2.1	1.00	0.93	0.72
	76 kDa[4]	29.4	25.0	30.8	26.0	6.5	6.5	6.5	6.5	0.86	0.49	0.98
3 Days	80 kDa[4]	7.8	9.8	9.6	8.4	2.3	2.3	2.3	2.3	0.93	0.87	0.50
	78 kDa[4]	26.2	28.2	28.4	27.4	2.9	2.9	2.9	2.9	0.81	0.87	0.62
	76 kDa[4]	65.8	62.4	62.2	63.6	4.9	4.9	4.9	4.9	0.81	0.84	0.63
7 Days	80 kDa[4]	ND	ND	1.2	2.6	1.5	1.5	1.5	1.5	0.22	0.64	0.64
	78 kDa[4]	13.8	14.6	17.0	19.6	4.4	4.4	4.4	4.4	0.37	0.70	0.84
	76 kDa[4]	87.2	85.4	82.0	77.8	5.0	5.0	5.0	5.0	0.23	0.56	0.81
Calpain-1, Sarcoplasmic[5]												
1 Hour	80 kDa[4]	100.0	100.0	100.0	100.0	ND	ND	ND	ND	ND	ND	ND
	78 kDa[4]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	76 kDa[4]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1 Day	80 kDa[4]	34.4	33.2	32.2	34.0	6.5	6.5	6.5	6.5	0.92	0.96	0.82
	78 kDa[4]	33.2	35.2	35.2	36.0	1.4	1.4	1.4	1.4	0.34	0.34	0.68
	76 kDa[4]	31.6	31.0	33.2	30.2	5.8	5.8	5.8	5.8	0.95	0.76	0.84
3 Days	80 kDa[4]	3.6	7.4	7.8	7.2	1.8	1.8	1.8	1.8	0.28	0.39	0.24
	78 kDa[4]	23.6	28.2	27.8	28.8	2.1	2.1	2.1	2.1	0.28	0.21	0.41
	76 kDa[4]	72.8	64.4	64.2	64.0	3.7	3.76	3.7	3.7	0.25	0.27	0.29
7 Days	80 kDa[4]	0.4	2.2	2.6	2.6	0.8	0.8	0.8	0.8	0.14	0.30	0.30
	78 kDa[4]	14.4	17.6	19.0	19.8	1.6	1.6	1.6	1.6	0.06	0.24	0.47
	76 kDa[4]	85.2	79.8	78.6	77.8	2.2	2.2	2.2	2.2	0.07	0.18	0.31

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg dry matter [DM]); SUPZN = CON + 60 mg Zn/kg DM (from ZnSO4) + 60 mg Zn/kg DM (from Zn–amino acid complex; Availa-Zn; Zinpro Corporation, Eden Prairie, MN). Fed for the entire 89-d trial.

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

[3]Whole muscle (WM) 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]Water-soluble protein fraction extracted calpain-1.

ND = not detectable; SEM = standard error of the mean; TRT = treatment.

been examined as indicators of postmortem proteolysis because of their known roles in structural integrity of skeletal muscle and contraction (Clark et al., 2002). Analysis of the whole muscle extract samples showed that supranutritional supplementation of Zn trended to have lesser whole muscle extract desmin degradation products at 3 and 7 d postmortem and lesser sarcoplasmic desmin degradation products at 3 and 14 d postmortem (Table 4; $P < 0.10$). Additionally, supranutritional Zn supplementation resulted in lesser troponin-T degradation at 3 and 14 d postmortem (Table 4; $P \leq 0.05$). This may be related to the observed trends for greater 78-kDa and lesser 76-kDa calpain-1 (Table 3; $P < 0.10$) in the sarcoplasmic fraction at 7 d postmortem of samples supplemented with supranutritional Zn. Reduced calpain-1 activity or earlier loss of calpain-1 activity will result in less postmortem protein degradation (Rowe et al., 2004). Previously, Zn introduced to muscle at levels higher than seen physiologically has been shown to have an inhibitory effect on calpain-1 activity (Koohmaraie, 1992). Thus, our results provide evidence that the relationship between

Table 4. Effect of supranutritional zinc (Zn) and ractopamine hydrochloride (RH) supplementation on whole-muscle (WM) extract desmin, soluble sarcoplasmic desmin, and troponin-T degradation products of beef *longissimus thoracis* muscle at different postmortem timepoints

	CON[1]		SUPZN[1]		SEM				*P* Value		
Item	NO[2]	RAC[2]	NO[2]	RAC[2]	CON-NO	CON-RAC	SUPZN-NO	SUPZN-RAC	ZN TRT	RAC TRT	ZN TRT × RAC TRT
WM Desmin Degradation[3]											
1 Hour	0.25	0.20	0.17	0.21	0.03	0.03	0.03	0.04	0.19	0.85	0.04
1 Day	0.45	0.22	0.29	0.26	0.06	0.06	0.07	0.07	0.37	0.05	0.14
3 Days	1.18	0.75	0.88	0.54	0.15	0.15	0.14	0.16	0.09	0.01	0.75
7 Days	2.86	2.03	1.63	1.31	0.21	0.21	0.22	0.21	<0.01	<0.01	0.12
14 Days	3.77	1.93	1.67	1.38	0.21	0.19	0.21	0.18	<0.01	<0.01	<0.01
Soluble Sarcoplasmic Desmin Degradation[4]											
1 Hour	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1 Day	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3 Days	0.44	0.14	0.18	0.05	0.08	0.08	0.08	0.07	0.03	<0.01	0.28
7 Days	0.66	0.31	0.12	0.40	0.15	0.16	0.14	0.13	0.07	0.74	<0.01
14 Days	1.41	1.19	0.36	0.45	0.23	0.23	0.23	0.22	<0.01	0.73	0.41
Troponin-T Degradation[5]											
1 Hour	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1 Day	1.29	0.68	1.09	0.65	0.24	0.24	0.23	0.26	0.64	0.04	0.74
3 Days	3.44	2.59	2.38	1.60	0.53	0.49	0.53	0.48	0.05	0.11	0.95
7 Days	4.93	4.56	3.71	4.55	0.61	0.55	0.59	0.54	0.16	0.59	0.18
14 Days	7.55	6.20	4.78	4.31	0.89	0.76	0.79	0.79	<0.01	0.27	0.59

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg dry matter [DM]); SUPZN = CON + 60 mg Zn/kg DM (from ZnSO4) + 60 mg Zn/kg DM (from Zn–amino acid complex; Availa-Zn; Zinpro Corporation, Eden Prairie, MN). Fed for the entire 89-d trial.

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

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

[4]Ratio of the densitometry units of the degraded 38-kDa band of the sample compared with the ~52-kDa band of the reference sample.

[5]Ratio of the densitometry units of the degraded 30-kDa band of the sample compared with the 30-kDa band of the reference sample.

ND = not detectable; SEM = standard error of the mean; TRT = treatment.

physiologically achievable Zn concentrations and calpain activity/autolysis needs to be characterized more fully. However, it must be kept in mind that, despite these observed trends of differences in calpain-1 autolysis at 7 d postmortem, this did not result in differences in WBSF values at 7 or 14 d postmortem between treatments.

Supplementation of RH resulted in lesser whole muscle extract desmin and troponin-T degradation products at 1 d postmortem (Table 4; $P \leq 0.05$) compared with samples not supplemented with RH. Lesser protein degradation at 1 d postmortem in samples supplemented with RH compared with samples not supplemented with RH could explain the increased WBSF values at 1 d postmortem in samples supplemented with RH compared with samples not supplemented with RH. Along with a higher pH at 6 h postmortem, a strong relationship between rate of pH decline, day-1 WBSF values, and day-1-postmortem proteolysis of desmin and troponin-T of whole muscle extracts is demonstrated in samples supplemented with RH. Correlation analysis further demonstrates this relationship between pH decline and postmortem proteolysis (Table 5). Sarcoplasmic calpain-1 76-kDa autolysis product demonstrated a moderately strong negative relationship with pH values at 3 h ($r = -0.58$; $P = 0.01$) and 6 h ($r = -0.61$; $P < 0.01$) postmortem. This relationship is strengthened with the shown correlation of pH values at 6 h postmortem trending to have a moderately strong negative correlation with whole muscle desmin ($r = -0.44$; $P = 0.06$) and troponin-T ($r = -0.39$; $P = 0.09$) degradation products at 1 d postmortem.

Additionally, RH supplementation resulted in less desmin degradation product in the whole muscle extract at 3 and 7 d postmortem and less appearance of the soluble desmin degradation product in the

sarcoplasmic fraction at 3 d postmortem (Table 4; $P < 0.01$). Less postmortem proteolysis in samples from RH-supplemented cattle could potentially also be due to greater calpastatin activity and inhibition of the calpains, resulting in less calpain-1 activity in muscle from cattle fed RH (Strydom et al., 2009).

The interaction of Zn and RH supplementation resulted in less whole muscle extract desmin degradation product at 1 h (Table 4; $P = 0.04$) and 14 d (Table 4; $P < 0.01$) postmortem compared with samples not supplemented with Zn or RH. Additionally, the interaction of Zn and RH supplementation resulted in less soluble sarcoplasmic desmin degradation products at 7 d (Table 4; $P < 0.01$) postmortem compared with samples not supplemented with Zn or RH. These observed differences in protein degradation further lend credence to the need to more fully understand the role of Zn and RAC fed in combination in muscle growth and early postmortem metabolism.

The relationship between postmortem protein degradation and instrumental tenderness values has been extensively studied (Huff-Lonergan et al., 1996; Hwang et al., 2004; Melody et al., 2004; England et al., 2012; Carlson et al., 2017b), and these data continue to demonstrate this strong relationship (Table 5). A moderately strong negative relationship was demonstrated between WBSF values at 3 d postmortem and the following: whole-muscle desmin degradation products at 1 h ($r = -0.49$; $P = 0.03$), 3 d ($r = -0.41$; $P = 0.07$), and 7 d ($r = -0.46$; $P = 0.04$) postmortem; sarcoplasmic calpain-1 autolysis at 7 d ($r = -0.49$; $P = 0.03$) postmortem; and whole-muscle troponin-T degradation products at 1 d ($r = -0.40$; $P = 0.08$), 3 d ($r = -0.52$; $P = 0.02$), and 14 d ($r = -0.39$; $P = 0.09$) postmortem. Furthermore, WBSF value at 7 d postmortem showed moderately and highly negative correlations with whole-muscle desmin degradation products at 7 d ($r = -0.48$; $P = 0.03$) and 14 d ($r = -0.59$; $P = 0.01$) postmortem, sarcoplasmic calpain-1 autolysis at 7 d ($r = -0.69$; $P < 0.01$) postmortem, and whole-muscle troponin-T degradation products at 7 d ($r = -0.62$; $P < 0.01$) and 14 d ($r = -0.50$; $P = 0.02$) postmortem. Warner-Bratzler shear force values at 14 d postmortem were moderately and highly negatively correlated with whole-muscle desmin degradation products at 3 d ($r = -0.39$; $P = 0.09$), 7 d ($r = -0.60$; $P = 0.01$), and 14 d ($r = -0.55$; $P = 0.01$) postmortem, sarcoplasmic calpain-1 autolysis at 7 d ($r = -0.57$; $P = 0.01$) postmortem, and troponin-T degradation products at 3 d ($r = -0.45$; $P = 0.05$), 7 d ($r = -0.70$; $P < 0.01$), and 14 d ($r = -0.71$; $P < 0.01$) postmortem. These results show the very strong

Table 5. Pearson correlations between postmortem pH, temperature (Temp), Warner-Bratzler shear force (WBSF), whole muscle desmin and troponin-T degradation products, and sarcoplasmic calpain-1 76-kDa autolysis[1]

	Desmin1 h	Desmin1 d	Desmin3 d	Desmin7 d	Desmin14 d	Calpain-11 d	Calpain-13 d	Calpain-17 d	Troponin-T1 d	Troponin-T3 d	Troponin-T7 d	Troponin-T14 d
pH 1 h	0.01	−0.1	−0.31	−0.36	*−0.39*	−0.22	−0.33	−0.27	−0.25	−0.23	−0.20	−0.16
pH 3 h	−0.06	−0.32	−0.37	−0.10	−0.16	**−0.58**	−0.47	0.05	−0.39	−0.21	0.04	−0.13
pH 6 h	0.07	*−0.44*	−0.32	0.06	0.02	**−0.61**	−0.31	0.25	*−0.39*	−0.13	0.27	0.09
pH 24 h	−0.30	−0.10	−0.16	−0.21	−0.30	0.01	0.12	0.07	−0.25	−0.22	−0.24	−0.15
Temp 1 h	−0.07	0.15	0.06	−0.11	−0.17	*0.42*	0.17	−0.34	0.12	−0.23	−0.28	−0.29
Temp 3 h	0.06	0.17	0.11	0.11	−0.09	−0.15	−0.12	0.04	0.08	0.13	−0.05	−0.01
Temp 6 h	−0.02	0.17	0.14	−0.08	−0.29	0.12	−0.10	−0.03	0.17	0.18	−0.12	−0.01
Temp 24 h	0.19	0.24	0.22	0.03	−0.04	0.20	0.07	−0.02	0.24	0.26	−0.14	0.04
WBSF 1 d	−0.26	−0.13	−0.23	−0.17	−0.05	−0.20	−0.22	−0.04	−0.24788	−0.11	0.14	−0.01
WBSF 3 d	**−0.49**	−0.24	*−0.41*	**−0.46**	−0.36	−0.04	−0.25	**−0.49**	*−0.40*	**−0.52**	−0.37	*−0.39*
WBSF 7 d	−0.33	0.01	−0.13	**−0.48**	**−0.59**	0.31	−0.00	**−0.69**	−0.04211	−0.28	**−0.62**	**−0.50**
WBSF 14 d	−0.33	−0.10	*−0.39*	**−0.60**	**−0.55**	0.12	−0.11	**−0.57**	−0.33	**−0.45**	**−0.70**	**−0.71**

[1]Significant correlations are bolded ($P \leq 0.05$). Trending correlations are italicized ($0.05 < P \leq 0.10$).

relationship between tenderness development and postmortem protein degradation that has been identified by the literature.

Conclusions

Supplementation of RH influenced pH at 6 h postmortem, possibly influencing the rate of proteolysis and WBSF values at 1 d postmortem. Supranutritional Zn supplementation trended to influence pH at 6 h postmortem and WBSF values at 1 d postmortem. Samples from cattle supplemented with supranutritional Zn alone trended to have the lowest pH at 6 h postmortem and the lowest WBSF value at 1 d postmortem compared with samples from cattle not supplemented with supranutritional Zn alone. Samples from cattle supplemented with the RH treatment alone had a higher pH at 6 h postmortem and the highest WBSF values at 1 d postmortem compared with samples not supplemented with RH alone. Samples from cattle supplemented with the RH treatment had less protein degradation at 1 d postmortem, which could explain the higher WBSF value in RH-supplemented cattle. Supplementation of supranutritional Zn did not influence indicators of protein degradation at 1 d postmortem. Due to the differences in postmortem pH decline at 6 h postmortem and WBSF values at 1 d postmortem, further investigation into the molecular explanations of these differences in pH decline and their impact on WBSF values and postmortem proteolysis is necessary in order to understand the impacts of supplementation of supranutritional Zn and RH in the diet.

Acknowledgments

This work is supported by the Agriculture and Food Research Initiative (AFRI) grant no. 2018-67015-27546/project accession no. 1015001 from the USDA National Institute of Food and Agriculture. The authors would like to thank Logan Johnson, Elizabeth Zuber, Taylor Wulf, Karli Durst, Michael Cropp, and the Iowa State University Meat Laboratory staff for assistance with this project.

Literature Cited

American Meat Science Association. 2012. AMSA meat color measurement guidelines. https://meatscience.org/publications resources/printed-publications/amsa-meat-color-measurement-guidelines. (Accessed 28 September 2020).

American Meat Science Association. 2016. AMSA guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. Version 1.02. https://meatscience.org/docs/default-source/publications-resources/amsa-sensory-and-tenderness-evaluation-guidelines/research-guide/amsa-research-guidelines-for-cookery-and-evaluation-1-02.pdf?sfvrsn=4c6b8eb3_2. (Accessed 17 March 2021).

Antonelo, D. S., J. F. M. Gomez, N. R. B. Consolo, M. Beline, L. A. Colnago, M. W. Schilling, X. Zhang, S. P. Suman, D. E. Gerrard, J. C. C. Baileiro, and S. L. Silva. 2020. Metabolites and metabolic pathways correlated with beef tenderness. Meat Muscle Biol. 4. https://doi.org/10.22175/mmb.10854.

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.

Bee, G., A. L. Anderson, S. M. Lonergan, and E. Huff-Lonergan. 2007. Rate and extent of pH decline affect proteolysis of cytoskeletal proteins and water-holding capacity in pork. Meat Sci. 76:359–365. https://doi.org/10.1016/j.meatsci.2006.12.004.

Bendall, J. R. 1978. Variability in rates of pH fall and of lactate production in the muscles on cooling beef carcasses. Meat Sci. 2:91–104. https://doi.org/10.1016/0309-1740(78)90010-4.

Bittner, C. J., G. I. Crawford, L. L. Berger, S. Holt, R. R. Pritchard, W. J. Platter, M. T. Van Koevering, N. A. Pyatt, and G. E. Erickson. 2016. Effect of ractopamine hydrochloride (Optaflexx) dose and duration on growth performance and carcass characteristics of finishing steers. J. Anim. Sci. 94:5382–5392. https://doi.org/10.2527/jas.2016-0807.

Bittner, C. J., M. A. Greenquist, D. B. Burken, A. L. Shreck, J. C. MacDonald, T. J. Klopfenstein, W. J. Platter, M. T. Van Koevering, N. A. Pyatt, and G. E. Erickson. 2017. Evaluation of ractopamine hydrochloride (Optaflexx) on growth performance and carcass characteristics of finishing steers across different feeding durations. J. Anim. Sci. 95:485. https://doi.org/10.2527/jas2016.0806.

Boehm, M. L., T. L. Kendall, V. F. Thompson, and D. E. Goll. 1998. Changes in the calpains and calpastatin during postmortem storage of bovine muscle. J. Anim. Sci. 76:2415–2434. https://doi.org/10.2527/1998.7692415x.

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, E. Huff-Lonergan, and S. M. Lonergan. 2017a. Proteomic features linked to tenderness of aged pork loins. J. Anim. Sci. 95:2533–2546. https://doi.org/10.2527/jas2016.1122.

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. 2017b. 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.

Clark, K. A., A. S. McElhinny, M. C. Beckerle, and C. C. Gregorio. 2002. Striated muscle cytoarchitecture: An intricate web of form and function. Annu. Rev. Cell Dev. Bi. 18:637–706. https://doi.org/10.1146/annurev.cellbio.18.012502.105840.

Ebarb, S. M., K. J. Phelps, J. S. Drouillard, K. R. Maddock Carlin, M. A. Vaughn, D. D. Burnett, J. A. Noel, C. L. Van Bibber-Krueger, C. B. Paulk, D. M. Grieger, and J. M. Gonzalez. 2017. Effects of anabolic implants and ractopamine-HCL on muscle fiber morphometrics, collagen solubility, and tenderness of beef *longissimus lumborum* steaks. J. Anim. Sci. 95:1219–1231. https://doi.org/10.2527/jas2016.1263.

England, E. M., K. D. Fisher, S. J. Wells, D. A. Mohrhauser, D. E. Gerrard, and A. D. Weaver. 2012. Postmortem titin proteolysis is influenced by sarcomere length in bovine muscle. J. Anim. Sci. 90:989–995. https://doi.org/10.2527/jas.2011-4278.

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 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. 2016b. 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. 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.

Genther-Schroeder, O. N., E. Huff-Lonergan, M. E. Branine, and S. L. Hansen. 2016c. Influence of zinc amino acid complex and ractopamine hydrochloride supplementation on the sarcoplasmic protein profile of finishing steers. J. Anim. Sci. 94:423. https://doi.org/10.2527/jam2016-0880.

Haase, H., and W. Maret. 2003. Intracellular zinc fluctuations modulate protein tyrosine phosphatase activity in insulin/insulin-like growth factor-1 signaling. Exp. Cell Res. 291:289–298. https://doi.org/10.1016/S0014-4827(03)00406-3.

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.

Hopkins, D. L., E. N. Ponnampalam, R. J. van de Ven, and R. D. Warner. 2014. The effect of pH decline rate on the meat and eating quality of beef carcasses. Anim. Prod. Sci. 54:407. https://doi.org/10.1071/an12314.

Hou, Q., C. Y. Zhang, W. G. Zhang, R. Liu, H. Tang, and G. H. Zhou. 2020. Role of protein S-nitrosylation in regulating beef tenderness. Food Chem. 306:125616. https://doi.org/10.1016/j.foodchem.2019.125616.

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.

Hwang, I. H., and J. M. Thompson. 2001a. The interaction between pH and temperature decline early postmortem on the calpain system and objective tenderness in electrically stimulated beef *longissimus dorsi* muscle. Meat Sci. 58:167–174. https://doi.org/10.1016/S0309-1740(00)00147-9.

Hwang, I. H., and J. M. Thompson. 2001b. The effect of time and type of electrical stimulation on the calpain system and meat tenderness in beef *longissimus dorsi* muscle. Meat Sci. 58:135–144. https://doi.org/10.1016/S0309-1740(00)00141-8.

Hwang, I. H., B. Y. Park, S. H. Cho, and J. M. Lee. 2004. Effects of muscle shortening and proteolysis on Warner-Bratzler shear force in beef *longissimus* and *semitendinosus*. Meat Sci. 68:497–505. https://doi.org/10.1016/j.meatsci.2004.04.002.

Kim, Y. H. B., R. D. Warner, and K. Rosenvold. 2014. Influence of high pre-rigor temperature and fast pH fall on muscle proteins and meat quality: A review. Anim. Prod. Sci. 54:375–395. https://doi.org/10.1071/AN13329.

Koohmaraie, M. 1992. Effect of pH, temperature, and inhibitors on autolysis and catalytic activity. J. Anim. Sci. 70:3071–3080. https://doi.org/10.2527/1992.70103071x.

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.

Lean, I. J., J. M. Thompson, and F. R. Dunshea. 2014. Meta-analysis of zilpaterol and ractopamine effects of feedlot performance, carcass traits and shear strength of meat in cattle. PLoS ONE. 9(12):e115904. https://doi.org/10.1371/journal.pone.0115904.

Lee, S., G. Channoit, R. McIntosh, D. A. Zvara, and Z. Xu. 2009. Molecular mechanism underlying Akt activation in zinc-induced cardioprotection. Am. J. Physiol.-Heart C. 297:H569–H575. https://doi.org/0.1152/ajpheart.00293.2009.

Li, Y. P., R. Liu, W. G. Zhang, Q. Q. Fu, N. Liu, and G. H. Zhou. 2014. Effect of nitric oxide on μ-calpain activation, protein proteolysis, and protein oxidation of pork during post-mortem aging. J. Agr. Food Chem. 62:5972–5977. https://doi.org/10.1021/jf501332d.

Liu, R., Y. Li, M. Wang, G. Zhou, and W. Zhang. 2016. Effect of protein S-nitrosylation on autolysis and catalytic ability of μ-

calpain. Food Chem. 213:470–477. https://doi.org/10.1016/j.foodchem.2016.06.104.

Liu, R., S. Lonergan, E. Steadham, G. Zhou, W. Zhang, and E. Huff-Lonergan. 2019. Effect of nitric oxide and calpastatin on the inhibition of μ-calpain activity, autolysis and proteolysis of myofibrillar proteins. Food Chem. 275:77–84. https://doi.org/10.1016/j.foodchem.2018.09.104.

Maddock Carlin, K. R., E. Huff-Lonergan, L. J. Rowe, and S. M. Lonergan. 2006. Effect of oxidation, pH, and ionic strength on calpastatin inhibition of μ- and m-calpain. J. Anim. Sci. 84:925–937. https://doi.org/10.2527/2006.844925x.

Marsh, B. B., T. P. Ringkob, R. L. Russell, D. R. Swartz, and L. A. Pagel. 1987. Effects of early-postmortem glycolytic rate on beef tenderness. Meat Sci. 21:241–248. https://doi.org/10.1016/0309-1740(87)90061-1.

Melody, J. L., S. M. Lonergan, L. J. Rowe, T. W. Huiatt, M. S. Mayes, and E. Huff-Lonergan. 2004. Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. J. Anim. Sci. 82:1195–1205. https://doi.org/10.1016/j.meatsci.2005.05.014.

National Academy of Sciences, Engineering, and Medicine. 2016. Nutrient requirements of beef cattle: 8th revised edition. The National Academies Press, Washington, D.C.

Nishimura, T., S. Fang, J. I. Wakamatsu, and K. Takahashi. 2009. Relationships between physical and structural properties of intramuscular connective tissue and toughness of raw pork. Anim. Sci. J. 80:85–90. https://doi.org/10.1111/j.1740-0929.2008.00600.x.

Park, J. H. Y., C. J. Grandjean, D. L. Antonson, and J. A. Vanderhoof. 1986. Effects of isolated zinc deficiency on the composition of skeletal muscle, liver, and bone during growth in rats. J. Nutr. 116:610–617. https://doi.org/10.1093/jn/116.4.610.

Pringle, T. D., C. R. Calkins, M. Koohmaraie, and S. J. Jones. 1993. Effects over time of feeding a beta-adrenergic agonist to wether lambs on animal performance, muscle growth, endogenous muscle proteinase activities, and meat tenderness. J. Anim. Sci. 71:636–644. https://doi.org/10.2527/1993.713636x.

Reinhardt, C., A. Millican, T. Wickersham, C. Larson, and M. Branine. 2019. Effect of zinc amino acid complexes on growth performance and carcass characteristics of finishing beef steers fed with or without ractopamine hydrochloride- a nine study pooled analysis. J. Anim. Sci. 97:159. https://doi.org/10.1093/jas/skz258.327.

Rowe, L. J., K. R. Maddock, S. M. Lonergan, and E. Huff-Lonergan. 2004. Oxidative environments decrease tenderization of beef steaks through inactivation of μ-calpain. J. Anim. Sci. 82:3254–3266. https://doi.org/10.2527/2004.82113254x.

Samuelson, K. L., M. E. Hubbert, and C. A. Löest. 2016. Effects of dietary urea concentration and zilpaterol hydrochloride on performance and carcass characteristics of finishing steers. J. Anim. Sci. 94:5350–5358. https://doi.org/10.2527/jas.2016-0875.

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 -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.

Taylor, R. 1990. Interpretation of the correlation coefficient: A basic review. J. Diagn. Med. Sonog. 6:35–39. https://doi.org/10.1177/875647939000600106.

Trotta, R. J., K. R. Maddock Carlin, and K. C. Swanson. 2019. Effects of ractopamine hydrochloride supplementation on feeding behavior, growth performance, and carcass characteristics of finishing steers. Translational Animal Science. 3:1143–1152. https://doi.org/10.1093/tas/txz114.

Warner, R. D., F. R. Dunshea, D. Gutzke, J. Lau, and G. Kearney. 2014. Factors influencing the incidence of high rigor temperature in beef carcasses in Australia. Anim. Prod. Sci. 54:363–374. https://doi.org/10.1071/AN13455.

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.

Wu, G., M. M. Farouk, S. Clerens, and K. Rosenvold. 2014. Effect of beef ultimate pH and large structural protein changes with aging on meat tenderness. Meat Sci. 98:637–645. https://doi.org/10.1016/j.meatsci.2014.06.010.

Zhang, C., R. Liu, A. Wang, D. Kang, G. Zhou, and W. Zhang. 2018. Regulation of calpain-1 activity and protein proteolysis by protein nitrosylation in postmortem beef. Meat Sci. 141:44–49. https://doi.org/10.1016/J.MEATSCI.2018.03.017.

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

	CON[1]		SUPZN[1]		SEM				*P*-value		
Item	NO[2]	RAC[2]	NO[2]	RAC[2]	CON-NO	CON-RAC	SUPZN-NO	SUPZN-RAC	ZNTRT	RACTRT	ZNTRT x RACTRT
Percent Purge[3]											
1 Day	2.3	2.6	2.4	2.6	0.3	0.3	0.3	0.3	0.79	0.39	0.75
3 Day	2.1	1.9	2.0	2.1	0.2	0.2	0.2	0.2	0.91	1.00	0.55
7 Day	1.7	1.7	1.6	1.7	0.2	0.2	0.2	0.2	0.83	0.94	0.81
14 Day	1.6	1.4	1.4	1.5	0.1	0.1	0.1	0.1	0.75	0.81	0.24
pH											
1 Day	5.46	5.47	5.48	5.46	0.03	0.03	0.03	0.03	0.79	0.91	0.63
3 Day	5.47	5.47	5.46	5.44	0.02	0.02	0.02	0.02	0.38	0.55	0.77
7 Day	5.57	5.57	5.57	5.53	0.01	0.01	0.01	0.01	0.14	0.06	0.18
14 Day	5.51	5.50	5.45	5.49	0.03	0.03	0.03	0.03	0.23	0.65	0.32
Marbling Score[4]											
1 Day	430	490	530	480	34	34	34	34	0.23	0.89	0.14
3 Day	450	500	540	440	31	31	31	31	0.56	0.36	0.04
7 Day	560	540	590	520	45	45	45	45	0.88	0.36	0.58
14 Day	520	480	560	550	39	39	39	39	0.17	0.57	0.68
Percent Cook Loss[5]											
1 Day	23.7	23.8	19.9	23.4	1.6	1.6	1.6	1.6	0.22	0.29	0.30
3 Day	22.8	25.1	20.9	21.5	1.1	1.1	1.1	1.1	0.03	0.23	0.47
7 Day	20.6	24.8	24.1	22.5	1.4	1.4	1.4	1.4	0.68	0.36	0.06
14 Day	23.8	23.6	26.1	25.8	0.8	0.8	0.8	0.8	0.02	0.73	0.98
L^*[6]											
1 Day	38.57	39.81	40.00	40.34	0.83	0.83	0.83	0.83	0.26	0.36	0.60
3 Day	39.61	41.55	40.03	41.20	0.79	0.79	0.79	0.79	0.96	0.07	0.64
7 Day	42.01	43.32	42.21	43.42	0.77	0.77	0.77	0.77	0.85	0.13	0.94
14 Day	41.22	42.38	42.13	43.33	0.69	0.60	0.60	0.60	0.16	0.09	0.98
a^*[6]											
1 Day	19.58	19.29	19.71	18.52	0.44	0.44	0.44	0.44	0.47	0.11	0.32
3 Day	21.33	21.16	21.78	20.23	0.62	0.62	0.62	0.62	0.70	0.19	0.29
7 Day	20.49	20.06	20.16	20.10	0.42	0.42	0.42	0.42	0.74	0.57	0.66
14 Day	21.70	21.24	21.94	21.83	0.37	0.37	0.37	0.37	0.29	0.47	0.65
b^*[6]											
1 Day	16.34	16.88	17.01	16.19	0.48	0.48	0.48	0.48	0.98	0.77	0.18
3 Day	18.16	18.92	18.50	18.87	0.45	0.45	0.45	0.45	0.75	0.23	0.66
7 Day	18.35	18.62	18.37	18.54	0.34	0.34	0.34	0.34	0.93	0.52	0.87
14 Day	19.18	19.36	19.39	19.89	0.39	0.39	0.39	0.39	0.36	0.40	0.69
Hue Angle[7]											
1 Day	39.85	41.16	40.79	41.13	0.38	0.38	0.38	0.38	0.25	0.05	0.22
3 Day	40.41	41.80	40.40	43.02	0.60	0.60	0.60	0.60	0.33	<0.01	0.32
7 Day	41.84	42.89	42.37	42.69	0.51	0.51	0.51	0.51	0.75	0.20	0.48
14 Day	41.45	42.34	41.47	42.34	0.58	0.58	0.58	0.58	0.99	0.16	0.99
Chroma[8]											
1 Day	25.51	25.63	26.03	24.60	0.63	0.63	0.63	0.63	0.69	0.32	0.24
3 Day	28.01	28.39	28.59	27.67	0.71	0.71	0.71	0.71	0.92	0.71	0.38
7 Day	27.50	27.37	27.28	27.35	0.48	0.48	0.48	0.48	0.80	0.95	0.84
14 Day	28.96	28.74	29.28	29.54	0.45	0.45	0.45	0.45	0.24	0.96	0.61

[1]CON = no supplemental Zn (analyzed 36 mg Zn/kg dry matter[DM]); SUPZN = CON + 60 mg Zn/kg DM from ZnSO4 + 60 mg Zn/kg DM from Zn-amino acid complex (Availa-Zn; Zinpro Corporation, Eden Prairie, MN). Fed for the entire 89 d trial.

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

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

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

[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] x 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: $(a^{*2}+b^{*2})^{1/2}$