

Effect of dietary zinc and ractopamine hydrochloride on pork chop muscle fiber type distribution, tenderness, and color characteristics^{1,2}

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ABSTRACT: A total of 320 finishing pigs (PIC 327 × 1050; initially 98 kg) were used to determine the effects of adding Zn to diets containing ractopamine HCl (RAC) on muscle fiber type distribution, fresh chop color, and cooked meat characteristics. Dietary treatments were fed for approximately 35 d and consisted of a corn–soybean meal–based negative control (CON), a positive control diet with 10 mg/kg of RAC (RAC+), and the RAC+ diet plus 75, 150, or 225 mg/kg added Zn from either ZnO or Availa-Zn. Loins randomly selected from each treatment ($n = 20$) were evaluated using contrasts: CON vs. RAC+, interaction of Zn level × source, Zn level linear and quadratic polynomials, and Zn source. There were no Zn source effects or Zn source × level interactions throughout the study ($P > 0.10$). Pigs fed RAC+ had increased ($P < 0.02$) percentage type IIX and a tendency for increased ($P = 0.10$) percent type IIB muscle fibers. Increasing added Zn decreased (linear, $P = 0.01$) percentage type IIA and tended to increase ($P = 0.09$) IIX muscle fibers. On d 1, 2, 3, 4, and 5 of display, pork chops from pigs fed the RAC+ treatment had greater ($P < 0.03$) L* values compared to the CON. On d 0 and 3 of display, increasing added Zn tended to decrease (quadratic, $P = 0.10$) L* values and

decreased (quadratic, $P < 0.03$) L* values on d 1, 2, 4, and 5. Pigs fed RAC+ had decreased ($P < 0.05$) a* values on d 1 and 4 of display and tended to have decreased ($P < 0.10$) a* values on d 0 and 2 compared to CON pork chops. Pork chops from the RAC+ treatment had a tendency for increased ($P < 0.08$) oxymyoglobin percentage compared to CON pork chops on d 1, 2, 4, and 5. On d 0, as dietary Zn increased in RAC+ diets, there was a decrease (linear, $P < 0.01$) in the formation of pork chop surface oxymyoglobin percentage. Metmyoglobin reducing ability (MRA) of pork chops on d 5 was decreased in the RAC+ group. Chops from pigs fed added Zn had increased (quadratic, $P < 0.03$) MRA on d 3 and 5 of the display period. There was a trend for increased (linear, $P = 0.07$) cooking loss with increasing Zn in RAC diets and treatments did not affect tenderness as measured by Warner-Bratzler shear force ($P > 0.07$). In conclusion, RAC+ diets produced chops that were lighter and less red but maintained a greater percentage of surface oxymyoglobin throughout a 5-d simulated retail display. Ractopamine reduced MRA at the end of the display period, but supplementing Zn to RAC diets restored MRA to near CON treatment levels at the end of the display period.

Key words: color, fiber type, pork, ractopamine

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doi:10.2527/jas2013-7318

INTRODUCTION

Ractopamine HCl (**RAC**; Paylean; Elanco Animal Health, Greenfield, IN) is a β -adrenergic agonist that is

approved to be fed to finishing swine during the final 20 to 40 kg of weight gain before harvest. Using this growth-promoting technology in pigs improves live performance and carcass characteristics (Apple et al., 2007). The trace mineral Zn has been suggested to increase the RAC response based on studies showing that added Zn will improve ADG and G:F (Patience and Chipman, 2011; Rambo et al., 2012).

While these studies provide justification to increase the amount of Zn in RAC-containing diets, no research

¹Contribution no.14-154-J from the KSAgric. Exp. Stn., Manhattan 66506.

²The authors express their appreciation to Elanco Animal Health (Greenfield, IN) for providing partial financial support for this experiment.

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Received October 28, 2013.

Accepted February 27, 2014.

has demonstrated the effect of these diets on fresh meat characteristics including color and cooked meat characteristics. Color is the single most important attribute consumers evaluate when making a purchasing decision (Hedrick et al., 1994; Mancini and Hunt, 2005). Feeding RAC can produce LM that is darker and less red (Apple et al., 2008). Tenderness constitutes the most important attribute consumers evaluate during their eating experience (Sanders et al., 2007). Meta-analysis indicates RAC increases shear force by 0.5 kg (Dunshea et al., 2005). In a subsequent meta-analysis, decreases in tenderness occurred in a dose-dependent manner as RAC inclusion level was increased (Apple et al., 2007). Both of these characteristics are influenced by muscle fiber type and the metabolic profile associated with these fibers (Ryu and Kim, 2005; Lee et al., 2010). Because RAC possesses a history of altering muscle fiber types (Aalhus et al., 1992; Depreux et al., 2002), the possible synergistic effect that RAC and Zn could elicit on muscle hypertrophy may alter the muscle's fiber type distribution to affect shelf life and tenderness. Therefore, the objective of this study was to evaluate the effects of adding Zn to RAC-containing diets on muscle fiber type distribution, fresh chop color, and cooked meat characteristics.

MATERIALS AND METHODS

Live Animal Management

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment.

A total of 320 finishing pigs (PIC 327 × 1050) with an average initial BW of 98 kg were housed at the Kansas State University Swine Teaching and Research Center (Manhattan, KS). The finishing barn was an environmentally controlled facility with 1.5 m² slatted-floor pens. Each pen was equipped with a dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. Two replications of the same barn were used. Within each replication, two 40-pen groups (24 barrow pens and 16 gilt pens or 16 barrow pens and 24 gilt pens per group) were subjected to the experimental treatments. Therefore, 4 groups of pigs were subjected to the following experimental procedures separately from one another.

Pens of pigs were allotted to 1 of 8 dietary treatments, with 2 pigs housed in each pen with a total of 20 pens per treatment. Dietary treatments consisted of a corn-soybean meal-based negative control diet formulated to 0.66% standardized ileal digestible (SID) Lys (CON); a positive control diet formulated to contain 0.92% SID lysine and 10 mg/kg of RAC (RAC+); the RAC+ diet plus 75, 150, or 225 mg/kg added Zn from ZnO; or the RAC+ diet plus 75, 150, or 225 mg/

kg added Zn from Availa-Zn (Zinpro, Eden Prairie, MN; Table 1). All diets contained 55 mg/kg Zn from ZnSO₄ provided by the trace mineral premix. Experimental diets were fed in meal form, and ZnO or Availa-Zn was added to the RAC diet at the expense of corn. Diets were fed for the last 41 d before slaughter for group 1 of the first barn replication and the last 35 d for the remaining groups of pigs in the barn replications.

Harvest and Sample Collection

At the completion of the feeding period, pigs were transported to the Kansas State University Meats Laboratory for harvest under Federal inspection. After a 24-h postslaughter chilling period, a 30.48-cm portion of the longissimus lumborum muscle (beginning at the 10th rib) from the left side of 1 randomly selected pig from each pen was collected for immunohistochemistry and fresh pork quality analysis. Additionally from this sample, 24-h pH was measured using a Hanna HI 99163 meat pH probe (Hanna Instruments, Smithfield, RI) inserted into the 11th through 12th rib interface, and a 2.54 cm thick chop was collected from this location to be used for immunohistochemical analysis. The remainder of the muscle sample was vacuum packaged and stored at 2 ± 3°C for 13 d postmortem.

Immunohistochemistry

A 1-cm² portion of muscle was collected from the geometric center of each chop designated for immunohistochemistry. After collection, the muscle was embedded in optimal cutting temperature tissue embedding media (Fisher Scientific, Pittsburgh, PA), frozen by submersion in supercooled isopentane, and stored at -80°C until analysis. For each sample, two 10-μm cryosections were collected on frost resistant slides (Fisher Scientific) and the methods of Gonzalez et al. (2008) were followed for immunodetection with modifications. Nonspecific antigen binding sites were inhibited by incubating cryosections in 5% horse serum and 0.2% TritonX-100 (Fisher Scientific) in PBS for 30 min. All sections were incubated with the following primary antibodies in blocking solution for 60 min: 1:50 α-dystrophin (Thermo Scientific, Waltham, MA), 1:10 supernatant myosin heavy chain, slow, IgG2b (BA-D5; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), 1:10 supernatant myosin heavy chain type 2A, IgG1 (SC-71; Developmental Studies Hybridoma Bank), and 1:10 supernatant myosin heavy chain type 2B, IgM (BF-F3; Developmental Studies Hybridoma Bank). After incubation, sections were washed with PBS 3 times for 5 min followed by incubation in the following secondary antibodies (1:1,000) in blocking solution for 30 min: Alexa-Fluor 488 goat anti-mouse IgM

Table 1. Diet composition (as-fed basis)^{1,2}

Item	Control	RAC ³
Ingredient, %		
Corn	83.06	74.24
Soybean meal, (46.5% CP)	15.22	23.97
Monocalcium P (21% P)	0.25	0.20
Limestone	0.75	0.78
Salt	0.35	0.35
Vitamin premix ⁴	0.075	0.075
Trace mineral premix ⁵	0.075	0.075
L-Lys HCl	0.15	0.15
D,L-Met	—	0.015
L-Thr	—	0.025
Phytase ⁶	0.075	0.075
Ractopamine HCl ⁷	—	0.05
Total	100	100
Calculated analysis, %		
Standardized ileal digestible (SID) amino acids, %		
Lys	0.70	0.92
Ile:Lys	71	70
Leu:Lys	179	158
Met:Lys	31	30
Met and Cys:Lys	65	60
Thr:Lys	63	64
Trp:Lys	19	19
Val:Lys	84	79
Total lysine, %	0.79	1.03
CP, %	14.3	17.6
ME, Mcal/kg ⁸	3.362	3.358
NE, Mcal/kg ⁸	2.301	2.269
SID lysine:ME, g/Mcal	2.08	2.74
Ca, %	0.41	0.44
P, %	0.39	0.42
Available P, %	0.21	0.21

¹Diets were fed in meal form for the duration of the experiment.

²Dietary treatments were obtained by replacing corn in the ractopamine HCl diet to achieve 75, 150, and 225 mg/kg added Zn from ZnO (Zinc Nacional S.A., Monterrey, Mexico) or Availa-Zn (Zinpro, Eden Prairie, MN).

³RAC = ractopamine HCl.

⁴Vitamin premix provided 3,307 IU vitamin A, 413 IU vitamin D₃, 13 IU vitamin E, 1.32 mg vitamin K, 11.6 µg vitamin B₁₂, 14.9 mg niacin, 8.27 mg pantothenic acid, and 2.48 mg riboflavin per kilogram of the complete diet.

⁵Trace mineral premix provided 16.53 mg Mn, 55.06 mg Fe, 55.06 mg Zn, 8.25 mg Cu, 0.15 mg I, and 0.15 mg Se per kilogram of the complete diet.

⁶Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 450.4 phytase units/kg, with a release of 0.1% available phosphorus.

⁷Provided 10 mg/kg of ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN).

⁸Values for ingredients were derived from NRC (1998).

for BF-F3 (Invitrogen, San Diego, CA), Alexa-Fluor 594 goat anti-mouse IgG1 for SC-71 (Invitrogen), Alexa-Fluor 633 goat anti-mouse IgG2b for BA-D5 (Invitrogen), and Alexa-Fluor 594 goat anti-rabbit heavy and light chains for α -dystrophin (Invitrogen). Additionally, 1:1,000 Hoechst Dye 33342 (Invitrogen) was used to identify all fiber associated nuclei. Finally, sections were washed for

three 5-min periods in PBS and then covered with 5 µL of 9:1 glycerol in PBS and were cover-slipped for imaging.

Cryosections were imaged using a Nikon Eclipse TI-U inverted microscope with 10x working distance magnification (Nikon Instruments Inc., Melville, NY). Four representative photomicrographs per section were captured using a Nikon DS-QiMc digital camera (Nikon Instruments Inc.) that was calibrated to the 10x objective. For myosin heavy chain fiber type data collection, a minimum of 2 photomicrographs per section (minimum 500 fibers per animal) were analyzed for isoform distribution using NIS-Elements Imaging Software (Basic Research, 3.3; Nikon Instruments Inc.). Fibers that were positive for the BA-D5 antibody were counted as type I fibers. Fibers strongly stained only for SC-71 or BF-F3 were labeled as type IIA and type IIB fibers, respectively. Fibers stained weakly for both SC-71 and BF-F3 were labeled as type IIX fibers.

Chop Cutting and Simulated Retail Display

At the conclusion of the 13-d aging period, loin muscles were removed from their packages and cut into five 2.54 cm thick chops. The first 4 chops were used for simulated retail display. Of these chops, the first 3 chops were used for d 0, 1, and 3 metmyoglobin reducing ability (MRA) analysis and the fourth chop was used for 5-d intact packaged chop surface color attributes including the collection of L*, a*, and b* values and spectral data for the calculation of surface myoglobin redox forms. The fourth chop was also used for d 5 MRA analysis. The final chop was immediately subjected to mechanical tenderness analysis by Warner-Bratzler shear force (WBSF).

All chops allocated to simulate retail display were placed on white 1S Styrofoam trays (Genpack, Glens Falls, NY) with a Dri Loc (Dri-Loc 50; Cryovac Sealed Air Corporation, Duncan, SC) absorbent pad and over-wrapped with polyvinyl chloride (PVC) film (MAPAC M [1,450 cm⁻³ · 645.2 cm⁻² · 24 h⁻¹, 72 gauge]; Bordon Packaging and Industrial Products, North Andover, MA). Chops were placed in coffin-style retail cases (Model DMF 8; Tyler Refrigeration Corporation, Niles, MI) at 3 ± 2°C. Cases were constantly illuminated with fluorescent lights (32 W Del-Warm White 3,000°K; Philips Lighting Company, Somerset, NJ) that emitted a constant 24 h case average intensity of 2,143 ± 113 lx. Every 12 h, chops were rotated from left to right and front to back in the cases to account for variation in temperature and light intensity. Absolute CIE L*, a*, and b* and spectral reflectance (400 to 700 nm) readings were taken at 3 locations on each 5-d retail display chop through the PVC packaging using a Hunter Lab Miniscan EZ spectrophotometer (Illuminant A, 2.54 cm diameter aperture, 10° observer; Hunter Associates Laboratory, Reston, VA). Absolute and spectral values from the 3 scans were averaged and reflec-

tance at 473, 525, 572, and 700 nm were used to calculate surface percentages of metmyoglobin and oxymyoglobin using the equations from Krzywicki (1979) as published in the American Meat Science Association color guidelines (AMSA, 2012).

Metmyoglobin Reducing Ability

The procedures of Gonzalez et al. (2009) and Watts et al. (1966) were followed for MRA with modifications. On the day of analysis, chops were pulled from the retail display case and cut into 5 by 5 cm portions that were indicative of the discoloration pattern for the entire chop. Each section was placed in a 400 mL beaker and oxidized in 100 mL of 0.3% sodium nitrite at $25 \pm 2^\circ\text{C}$ for 20 min. After the samples were blotted of excess solution, they were vacuum packaged in 25.4 by 30.5 cm Prime Source Vacuum Pouches (76.2 μm standard barrier; Bunzl Processor Division, Koch Supplies, Kansas City, MO) that possess an oxygen transmission rate of $4.5 \text{ cm}^3 \cdot \text{cm}^{-2} \cdot 24 \text{ h}^{-1}$ at 23°C and 65% relative humidity. Reflectance measurements (400 to 700 nm) were collected initially after vacuum packaging and every 30 min for 2 h using a Hunter Lab Miniscan EZ spectrophotometer (Illuminant A, 2.54 cm diameter aperture, 10° observer; Hunter Associates Laboratory). Metmyoglobin was calculated as described above. Metmyoglobin reducing ability was calculated as (observed decrease in metmyoglobin concentration/initial metmyoglobin concentration) \times 100.

Warner-Bratzler Shear Force Analysis

The American Meat Science Association (AMSA, 1995) guidelines for instrumental cooked meat tenderness were followed for shear force analysis. Fresh cut chops were weighed and a thermocouple wire (30-gauge and constantan; Omega Engineering, Stamford, CT) was inserted into the geometric center of each chop for internal temperature monitoring using a Doric Minitrend 205 monitor (VAS Engineering, San Francisco, CA). Chops were cooked on electric, open-hearth Farberware grills (Model 450-A; Yonkers, NY) to an internal temperature of 35°C and then flipped and cooked to a final internal temperature of 71°C . After cooked chops were chilled overnight at $7 \pm 1^\circ\text{C}$, six 1.27 cm diameter cores were extracted from each chop parallel to the muscle fiber orientation. Each core was sheared once through the center of the core perpendicular to the muscle fiber orientation using an Instron Model 5569 Testing Machine (Instron, Canton, MA) with a Warner-Bratzler shear head attached (100 kg compression load cell and crosshead speed of 250 mm/min). Cooking loss was determined by measuring the difference in chop weight before and after cooking and dividing by precooked chop weight.

Statistics

All data were analyzed as a generalized randomized complete block design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pen as the experimental unit and animal/chop as the observational unit. For cooked meat analysis and fiber isoform distribution, dietary treatment served as the fixed effect while gender within group within barn was included as random effects. Contrast statements consisted of 1) negative control vs. positive control RAC diet, 2) interaction between increasing Zn level and Zn source, 3) increasing Zn linear and quadratic polynomials, and 4) added Zn from ZnO vs. Availa-Zn. For shelf-life analysis, the statistical structure was the same except for day of display and the day \times treatment interaction served as fixed effects in addition to dietary treatment. Day of display \times dietary treatment interaction was evaluated by the following contrast: 1) interaction between negative control vs. positive control RAC diet and day of display, 2) interaction between increasing Zn level and day of display, 3) interaction between Zn source and day of display, and 4) interaction between increasing Zn level, Zn source, and day of display. Day of display also served as the repeated measure with chop as the subject. Statistical significance was determined at $P < 0.05$ and trends or tendencies were determined at $0.05 > P > 0.10$.

RESULTS

For all dependent variables observed in the study, there was no Zn source effect or an interaction between Zn source and level ($P > 0.10$). Therefore, all data presented will combine the Zn treatment groups by the level of supplementation, which consists of 75 (**75Zn**), 150 (**150Zn**), and 225 mg/kg (**225Zn**) Zn.

Using immunohistochemical techniques, the effect of the dietary treatments on myosin heavy chain isoform distribution was evaluated (Fig. 1). Our data indicated that the percentage of type I muscle fibers were not affected by RAC+ or RAC diets with added Zn ($P > 0.10$). There was no difference ($P = 0.16$) in the percentage of type IIA fibers when comparing CON and RAC+ treated muscles. However, there was a decrease (linear, $P = 0.01$) in percentage of type IIA fibers as Zn concentration increased. Loin muscle samples from pigs fed the RAC+ treatment had a decreased ($P = 0.02$) percentage of type IIX muscle fibers compared to the CON. There was only a tendency for an increased (linear, $P = 0.09$) percentage of type IIX fibers when supplemental Zn was added to the RAC diet. For type IIB fibers, pigs in the RAC+ treatment had a tendency to possess more ($P = 0.10$) fibers than CON pigs. Finally, adding supplemental Zn to the RAC diet did not affect ($P > 0.10$) the percentage of type IIB muscle fibers in the loin.

Pork chops from all treatment groups were displayed under simulated retail conditions for 5 d and daily objec-

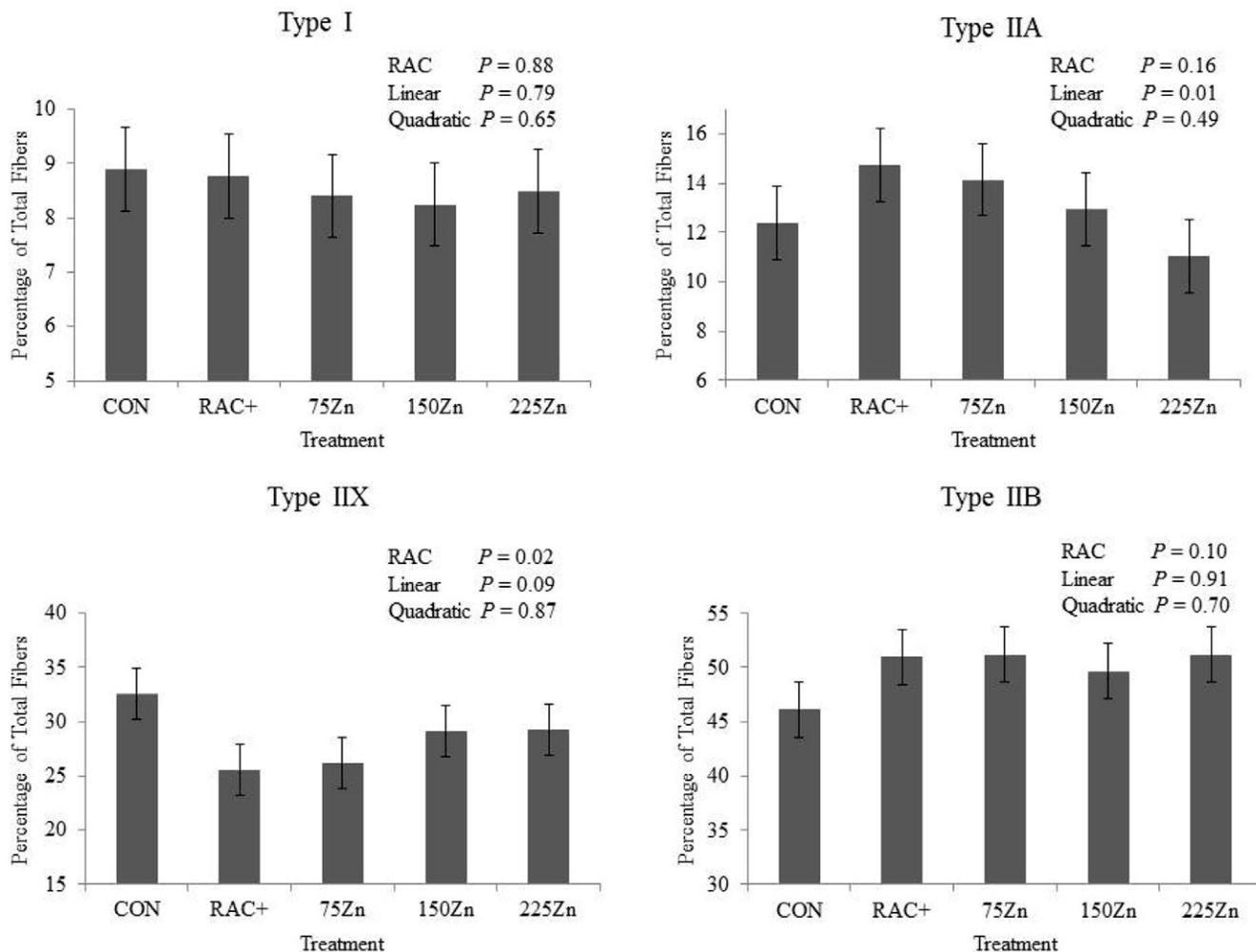


Figure 1. Tenth rib longissimus lumborum myosin heavy chain isoform distribution of pigs fed a basal diet containing 0 mg/kg ractopamine HCl (CON), pigs supplemented 10 mg/kg ractopamine HCl (RAC+), and pigs supplemented 10 mg/kg ractopamine HCl and 75 (75Zn), 150 (150Zn), or 225 mg/kg (225Zn) of zinc. RAC = ractopamine HCl.

tive measures of pork color were collected. For L^* , a^* , and b^* values (Fig. 2), only a^* and b^* values were affected by day of display (quadratic, $P < 0.05$). Pork chops of pigs from the RAC+ treatment did not differ ($P > 0.10$) in L^* values compared to CON pork chops on d 0 of the display period. On d 1, 2, 3, 4, and 5, pork chops from pigs fed the RAC+ treatment had greater L^* values compared to the CON pork chops ($P < 0.03$). On d 0 and 3 of display, increasing the Zn content of the diet resulted in a trend for lower L^* values (quadratic, $P = 0.10$). In addition, on d 1, 2, 4, and 5 of the display period, increasing Zn reduced the L^* values (quadratic, $P < 0.03$). Of the Zn treatment groups, pork chops from pigs in 150Zn treatment possessed the lowest L^* values over the entire display period. Pork chops from RAC+ pigs possessed lower a^* values compared to CON pork chops on d 1 and 4 of display ($P < 0.05$). However, RAC+ chop a^* values tended to be lower on d 0 and 2 of display ($P < 0.10$). On d 4 of display, a^* values were greater (quadratic, $P = 0.04$) as Zn was added to the diet, with 150Zn chops ob-

taining in the greatest a^* value. For all other display days, increasing dietary Zn in the diet did not affect a^* values ($P > 0.10$). On d 0 of display, b^* values of pork chops from RAC+ pigs were not different ($P > 0.10$) from CON pigs. However, for the remainder of the display period, chops from RAC+ pigs possessed lower b^* values than CON pigs ($P < 0.04$). On d 1 of display, adding Zn to the diet decreased (quadratic, $P = 0.03$) b^* values, with the lowest response detected in the Zn75 group. On d 2 and 4, supplemental Zn tended to decrease b^* values, with Zn150 exhibiting the lowest value ($P < 0.09$).

The objective measures of chop surface oxymyoglobin and metmyoglobin percentages indicate that there was a day effect on both redox forms ($P < 0.001$; Fig. 3). For oxymyoglobin, there was an increase (quadratic, $P < 0.001$) in chop surface percentages from d 0 to d 1, but thereafter the surface percentage of oxymyoglobin decreased. On d 0 of the display period, the percentage of oxymyoglobin formed on the surface of chops was not different ($P = 0.63$) between CON and RAC+ treated

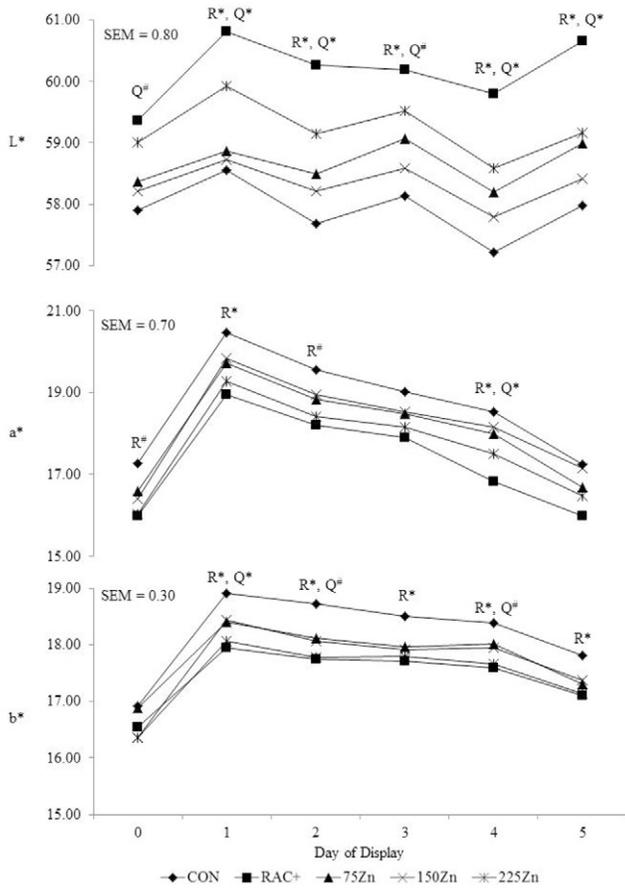


Figure 2. Surface L*, a*, and b* values of loin chops from pigs fed a basal diet containing 0 mg/kg ractopamine HCl (CON), pigs supplemented 10 mg/kg ractopamine HCl (RAC+), and pigs supplemented 10 mg/kg ractopamine HCl and 75 (75Zn), 150 (150Zn), or 225 mg/kg (225Zn) of zinc. L* = lightness (0 = black and 100 = white), a* = redness (−60 = green and 60 = red), and b* = yellowness (−60 = blue and 60 = yellow). R designates a ractopamine HCl effect and Q designates a quadratic zinc effect. The superscript * indicates a significant effect ($P \leq 0.05$), while the superscript # indicates marginal significance ($P \leq 0.10$).

pork chops. However, on the same day of display, as dietary Zn increased from 0 to 225 mg/kg in RAC diets, there was a decrease (linear, $P < 0.01$) in the formation of pork chop surface oxymyoglobin percentage. For the remaining days of display, RAC+ treated pork chops had a tendency for greater surface oxymyoglobin percentage compared to CON pork chops on d 1, 2, 4, and 5 ($P < 0.08$). For metmyoglobin surface percentage, as the day of display increased, the surface percentage of metmyoglobin increased (quadratic, $P < 0.001$); however, inclusion of a dietary RAC or supplemental Zn did not affect chop surface metmyoglobin accumulation ($P > 0.10$).

As expected, all chops exhibited a reduction ($P < 0.0001$) in MRA as the day of display increased (Fig. 4). At the beginning of the display period, chop MRA ranged from 52 to 56%. When compared to the CON treatment, RAC+ did not affect MRA on this day or d 1 and 3 of the display period ($P > 0.10$). By d 5 of display, chop MRA ranged from 31 to 42%. Inclusion of RAC in the diet reduced ($P < 0.001$) MRA compared

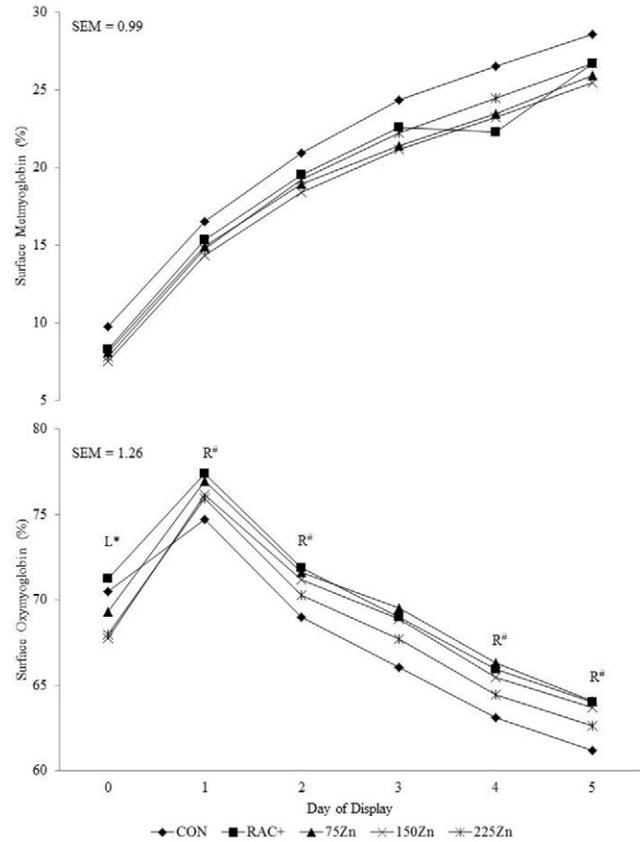


Figure 3. Surface oxymyoglobin and metmyoglobin percentages of loin chops from pigs fed a basal diet containing 0 mg/kg ractopamine HCl (CON), pigs supplemented 10 mg/kg ractopamine HCl (RAC+), and pigs supplemented 10 mg/kg ractopamine HCl and 75 (75Zn), 150 (150Zn), or 225 mg/kg (225Zn) of zinc. R designates a ractopamine HCl effect and L designates a linear zinc effect. The superscript * indicates a significant effect ($P \leq 0.05$), while the superscript # indicates marginal significance ($P \leq 0.10$).

to the CON pork chops. While supplemental Zn did not affect MRA on d 0 and 1 ($P > 0.10$) of display, as the level of dietary Zn was increased, there was an increase (quadratic, $P < 0.03$) in MRA on d 3 and 5 of the display period. Seventy-five milligrams per kilogram of added Zn was sufficient to maximize the MRA of RAC-treated pork chops on d 3 of display while 150 mg/kg of added Zn resulted in the greatest MRA on d 5.

Chops from RAC+ pigs did not differ in pH, cooking loss, or shear force values when compared to CON chops (Table 2; $P > 0.10$). However, there was a trend for increased (linear, $P = 0.07$) cooking loss as dietary Zn increased from 0 to 225 mg/kg in RAC diets.

DISCUSSION

Our method of muscle fiber type assignment is in agreement with Lefaucheur et al. (2002), who used the same set of antibodies in the LM of the pig. Similar to our findings, the authors reported that SC-71 recognized both type IIA and IIX fibers, with the IIA fibers staining more intensely than the IIX fibers. In addition, our fiber

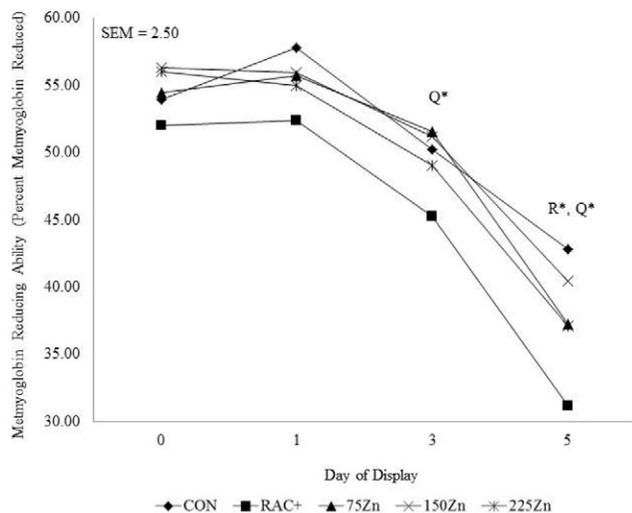


Figure 4. Metmyoglobin reducing ability of loin chops pigs fed a basal diet containing 0 mg/kg ractopamine HCl (CON), pigs supplemented 10 mg/kg ractopamine HCl (RAC+), and pigs supplemented 10 mg/kg ractopamine HCl and 75 (75Zn), 150 (150Zn), or 225 mg/kg (225Zn) of zinc. R designates a ractopamine HCl effect and Q designates a quadratic zinc effect. The superscript * indicates a significant effect ($P \leq 0.05$).

isoform distribution pattern was similar to the distribution reported by the authors with a greater percentage of type IIB fibers (46 to 50%), a moderate percentage of type IIX fibers (25 to 32%), and low percentages of type I (8%) and IIA (14 to 11%) fibers.

Numerous reviews document the effect that muscle fiber distribution can have on fresh meat quality and color (Klont et al., 1998; Lee et al., 2010). In our study, we found that feeding 10 mg/kg of RAC to pigs during the final 35 d of feeding did not have an effect on type I or type IIA fiber percentage but decreased the percentage of type IIX fibers while tending to increase the percentage of type IIB fibers. Using histological techniques, Aalhus et al. (1992) reported that RAC supplemented at 20 mg/kg did not affect the percentage of red (type I) fibers, but the percentage of intermediate fibers (type IIA/X) were reduced while white fibers (type IIB) were increased. Depreux et al. (2002) also found that type I and IIA fibers in the LM were unaffected by either 20 or 60 mg/kg of RAC supplementation as detected by ELISA. In agreement with the current study, the researchers reported that there was a strong significant correlation ($R = -0.768$) between decreases in type IIA/X fibers and increases in type IIB fibers. Gunawan et al. (2007) also reported that RAC supplemented at 20 mg/kg increased the mRNA expression of type IIB fibers at the expense of type IIX fibers, while type I expression was unaffected. Interestingly, the authors found that type IIA expression decreased 96 h after initial RAC administration and continued to decrease for approximately 1 wk. However, by the end of the 4-wk trial, type IIA expression returned to presupplementation levels. In the current study we dem-

onstrate that increasing Zn in the RAC-containing diets decreased the type IIA isoform throughout the feeding period, which tended to correspond to increases in type IIX fibers. Therefore, this data indicate that Zn supplementation could have inhibited the reestablishment of the presupplementation type IIA fiber pool, as seen by Gunawan et al. (2007), which catalyzed a decrease in the percentage of type IIA while increasing type IIX fibers.

The literature documents many of the live production and carcass characteristic advantages of feeding RAC and these advantages serve as the main incentive for pork producers to use RAC in their operations (Apple et al., 2007; Bohrer et al., 2013). However, the literature contains mixed and variable results when examining the effect of RAC on cooked chop tenderness and other fresh meat quality characteristics. In the present study, we observed that neither RAC nor Zn supplementation elicited an effect on 24-h pH. Others have demonstrated that increasing the percentage of type IIB fibers in the muscle negatively impacts the extent of pH decline (Ryu et al., 2006) and that this decline is due to significantly greater glycogen and lactate concentrations produced by the type IIB fibers in the early postmortem period (Choe et al., 2008). Therefore, the effect of the RAC induced fiber shift toward more type IIB fibers and this effect on pH development becomes a major concern. Since we did not experience ultimate pH development differences between treatments, we attribute this finding to our RAC+ treatment only increasing the percentage of type IIB fibers by less than 5%. The lack of a RAC effect on ultimate pH reported here is in agreement with studies examining the RAC response on numerous variables including breed (Stoller et al., 2003), heavy weight/late finishing pigs (Fernández-Duenas et al., 2008; Kutzler et al., 2011), or pigs raised under commercial conditions (Athayde et al., 2012). Of the numerous studies that have found a significant effect of RAC on ultimate pH (Carr et al., 2005; Apple et al., 2008; James et al., 2013), pH values were increased by 0.07 to 0.08. The lack of 24-h pH effects indicates that our treatments did not affect postmortem ultimate pH; therefore, this demonstrates that the meat quality attributes measured in the study are independent of treatment catalyzed ultimate pH differences.

In a survey of consumers, Sanders et al. (2007) found that pork tenderness was important to 57% of consumers and these consumers were willing to pay a US\$0.82/kg premium for a guaranteed juicy and tender product. Hence, efficiently producing meat products that possess the peak palatability attributes is the goal of livestock producers. There are no data available that demonstrate the effect of Zn on tenderness and the available data evaluating the impact of β -agonists are inconsistent. When feeding β -agonists, the poor tenderness of products harvest-

Table 2. Least squares means of pork longissimus lumborum chop cooked meat characteristics from pigs supplemented ractopamine HCl (RAC) and 3 levels of dietary zinc

Item	Control	RAC ¹	Zinc, mg/kg ²			SEM	P-value		
			75	150	225		RAC	Zn linear	Zn quadratic
Cooking loss, %	24.74	23.54	25.06	24.60	25.63	0.98	0.30	0.07	0.70
Shear force, kg	3.56	3.55	3.76	3.63	3.73	0.14	0.97	0.44	0.59
pH ³	5.44	5.43	5.44	5.46	5.44	0.02	0.89	0.67	0.43

¹10 mg/kg of ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN) fed during the experiment.

²Dietary treatments were obtained by replacing corn in the ractopamine HCl diets to achieve 75, 150, and 225 mg/kg added Zn from ZnO (Zinc Nacional S.A., Monterrey, Mexico) or Availa-Zn (Zinpro, Eden Prairie, MN).

³pH collected at 24 h postmortem.

ed from animals fed these compounds is attributed to 2 mechanisms. The first mechanism is the lack of postmortem proteolytic activity (Wheeler and Koohmaraie, 1992), while the second is large increases in muscle fiber hypertrophy (Carr et al., 2005). Numerous studies in swine indicate that RAC supplementation can decrease tenderness as measured by WBSF by as much as 29% (Uttaro et al., 1993; Herr et al., 2001; Athayde et al., 2012), while other studies report no RAC effect on tenderness (Stoller et al., 2003; Apple et al., 2008; Kutzler et al., 2011). Stoller et al. (2003) reported that while they detected a significant RAC effect on WBSF, their trained sensory panel was unable to identify a significant RAC influence on tenderness and juiciness. Carr et al. (2005) found that when feeding RAC at 10 or 20 mg/kg, both WBSF and sensory panel tenderness scores indicated that RAC increased chop toughness. Our data falls into the category of studies that were unable to detect a RAC effect on tenderness. Even though RAC+ increased the cross-sectional area of type IIA and IIX fibers (Paulk et al., 2014), aging the loins for 2 wk may have negated the effect these larger fibers had on tenderness. Xiong et al. (2006) reported that subjecting pork from RAC-fed animals for extended aging duration improved pork tenderness, which suggests that there is a sufficient amount of postmortem proteolytic activity in RAC-supplemented pigs to improve tenderness.

A concerning trend we detected in our study was that when Zn was added to RAC diets, moisture retention during cooking tended to be reduced. As dietary Zn increased, cook loss increased by up to 2.09% in the 225Zn treatment group. Therefore, this increase in moisture loss during cooking could have a negative effect on consumer palatability due to the reduction of moisture in the cooked product and the loss of the benefits associated with increased juiciness. Our major concern is that with this loss of moisture, an increased percentage an increased percentage of pork from pigs fed both RAC and Zn could be perceived by the consumer as not being tender.

The literature contains a limited amount of comprehensive studies that document the effect that RAC or Zn elicit on fresh chop color during a simulated re-

tail display period. Apple et al. (2008) conducted a 5-d retail shelf-life study and reported that there was no interaction between RAC, day of display, and dietary fat source. Therefore, the authors reported only the main effects, which indicated that over the 5-d retail display period, RAC chops were slightly darker and less red and yellow than control chops. Our study produced RAC+ chops that were divergent in fresh color characteristics when compared to CON chops. Compared to the data of Brewer et al. (1998) and Joo et al. (1995), who used different illuminants that the current study (F and D65, respectively), all chops in the current study had greater L* values than chops that were categorized as pale, soft, and exudative (PSE). Specifically, compared to Brewer et al. (1998), chops from RAC+ pigs had L* values that were near values of PSE chops (65.91), while CON chop values were more near normal chop values (53.09). Chops from RAC+ pigs were lighter and less yellow in color on d 1 through 5 of display and less red on d 0, 1, 2, and 4 of display. Additionally, RAC+ chops possessed more surface oxymyoglobin on all days of display except d 0 and 3. These findings are consistent with studies that indicate that RAC-supplemented chops are lighter than nonsupplemented chops when color is measured at 24 h postmortem (Armstrong et al., 2004; Leick et al., 2010). Bergstrom (2011) did not find a RAC-induced reduction in the lightness of chops but found that RAC supplementation reduced chop a* and b* values over a 6-d shelf-life period. However, Rickard et al. (2012) found that RAC increased redness values during 7 d of display when products were stored at 4°C for 30 d or -20°C for 60 d. But when chops were immediately displayed after a 24-h chill, RAC decreased chop redness compared to the non-RAC chops. Because our chops were aged between the 24-h and 30-d storage period and RAC decreased redness, it appears that the RAC effect on objective color characteristics is dependent on length of storage.

Carr et al. (2005) hypothesized that the LM of RAC-supplemented pigs possessed lower a* values because of the shift of intermediate fibers to white fibers. The authors also concluded that the lower a* values were an

indication of reduced oxymyoglobin formation on the surface of the chops, which was a result of rapid fiber hypertrophy and dilution of myoglobin in the muscle. In our study, we also found that RAC+ chops tended to possess more type IIB fibers than CON chops. However, we found that surface oxymyoglobin content was greater on the surface of RAC+ chops. This could be due to RAC+ chops containing less mitochondria because of their greater type IIB isoform distribution. When muscle possesses a copious amount of mitochondria, myoglobin must compete with these organelles for oxygen to consume, which results in less oxymyoglobin formation (Klont et al., 1998). Therefore, since the RAC+ chops possessed a fiber type distribution that favored the presence of less mitochondria, more oxygen was available for consumption by the myoglobin in the muscle, which increased the formation of oxymyoglobin. This hypothesis is supported by work in beef that demonstrates that the inside semimembranosus muscle (greater surface oxymyoglobin content) possesses a lower oxygen consumption rate when compared to the outside semimembranosus (Sammel et al., 2002).

An interesting trend we detected is that addition of Zn to the RAC diets seemed to shift chop color characteristics away from RAC+ values and toward CON chops values. Quadratic Zn effects were detected on d 3 for a^* values, d 1 through 5 for L^* values, and d 1, 2, and 4 for b^* values. For d 0 oxymyoglobin percentage, there was a linear Zn effect on this day, with increasing Zn in the diet resulting in a decrease in oxymyoglobin percentage. Ma et al. (2012) reported that when Zn was deleted from the swine diet, a^* and b^* values were reduced. While the authors did not give an explanation behind these value drops, our results show that Zn supplementation to RAC diets can have a restorative effect on objective color values. Additionally, since Zn supplementation decreased the amount of type IIA fibers resulting in more IIX and IIB fibers, we believe this finding is the result of the same mechanism described previously.

The same trend of Zn supplementation restoring the color characteristics of RAC+ chops to that of CON chops can also be seen in the MRA data. The RAC effect on MRA was not detected until d 5 of the display period when RAC+ chops possessed 11.6% less MRA than CON chops. This finding can be a function of the type IIB fiber type shift and reduction of mitochondria that also reduces the amount of NADH in the muscle (Howlett and Willis, 1998). Thus, the MRA of the muscle is limited because there is less NADH to reduce metmyoglobin formation (Mancini and Hunt, 2005). Gonzalez et al. (2009) conducted a RAC study in beef that looked at the same shelf-life characteristics as the current trial. In contrast to this study, the authors did not find a RAC effect on oxymyoglobin and metmyoglobin formation

or MRA of LM steaks displayed for 5 d. However as was seen in our study, at the end of the display period, steaks supplemented with RAC began to have reduced MRA. While the difference between RAC+ and CON MRA detected at d 5 did not translate to increases in chop surface metmyoglobin formation, extending the display period could demonstrate that the RAC-induced reduction in MRA may result in greater metmyoglobin formation on the surface of these chops. When additional Zn was added to the diet, there was a quadratic Zn effect in which adding 150 mg/kg of Zn maximized MRA by 9.2% over RAC+ chops. This same effect was seen at d 3, where adding 75 mg/kg of Zn to the diet increased MRA by 6.3% over RAC+ chops. While the exact mechanism is unknown, we hypothesize that the IIA fiber type shift could play a crucial role in establishing the optical oxygen consumption rate and MRA as hypothesized by McKenna et al. (2005). Additionally, if extending the display period proves that RAC reduces color stability during extended display, Zn supplementation can serve as a countermeasure to these effects as indicated by the ability of the Zn treatments to restore MRA and a^* values close to control values.

Conclusion

Feeding pigs 10 mg/kg of RAC during the final 35 d before slaughter decreased the amount of type IIX fibers while tending to increase the percentage of type IIB fibers in the longissimus lumborum. Supplementing the RAC diets with dietary Zn above the NRC requirement decreased the percentage of type IIA fibers and tended to increase the percentage of type IIX fibers. These fiber shifts had effects on meat color characteristics. Ractopamine HCl produced chops that were lighter and less red but maintained a higher percentage of surface oxymyoglobin throughout a 5-d simulated retail display. While RAC improved these shelf-life characteristics, it reduced MRA at the end of the display period. Supplementing Zn to RAC diets restored MRA to near CON treatment levels at the end of the display period, which is most important to retailers. Zinc supplementation tended to increase chop cook loss, which may impact sensory attributes of the chops and should be explored further.

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