EFFECT OF VITAMIN B_3 DOSE ON FATIGUED BARROW CARCASS CHARACTERISTICS AND LOIN CHOP COLOR STABILITY

by

MORGAN GRAVELY

(Under the Direction of John Michael Gonzalez)

ABSTRACT

One hundred barrows were supplemented one of five different levels of a vitamin B_3 analog, nicotinamide riboside (NR), beginning 11 d prior to a fatigue inducing event. Barrows were then harvested, fabricated, and loins from each were aged in vacuum bags for 10 d. Chops from those loins were used in an 8-d simulated retail display and other laboratory assays to determine the effects of NR supplementation on fatigued barrow color stability and related measures. Supplementation of NR did not produce any positive effects on color stability, metmyoglobin reducing ability, oxygen consumption, carcass yields, postmortem pH, water holding capacity, proximate composition, muscle fiber type, muscle fiber cross-sectional area. Day of display did affect all measures during the simulated retail display period of the study.

INDEX WORDS: color stability, fatigued pig, nicotinamide riboside, pork quality, vitamin B_3

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DEDICATION

I would like to dedicate this to my incredibly supportive family: Marianne Cobb, Emerson Gravely, Jr., Bill and Terry Cobb, Randall and Celia Evans, and Dylan Davis; and to my grandparents who are watching from above: Betty and E. Hooper Gravely.

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First, I would like to thank my major professor, Dr. John Michael Gonzalez, and my committee members, Dr. Alexander Stelzleni and Dr. Dean Pringle. The three of you always encouraged an environment of constant learning. Your guidance was irreplaceable, and for that I will always be grateful. Secondly, I cannot thank the wonderful laboratory managers here at the University of Georgia Department of Animal and Dairy Science. To Mr. Ryan Crowe, thank you for always helping me, putting up with our crew, lending your expertise and your facilities. To Ms. Gina McKinney, thank you for always being so kind and understanding. You put up with us and never complained, and were always willing to help when needed. To Ms. Savannah Cheek, thank you for putting up with us!

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I would like to thank my parents - Marianne Cobb and Emerson Gravely, who support and understand me through anything I want to do. Thank you to my grandparents, Randall and Celia Evans, who have been my fairy godparents since I was a little girl, and always encouraged my education. Thank you to my grandparents, Bill and Terry Cobb, who supported and encouraged me more than I could have ever asked for through this degree and gave me a place to call home away from home.

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CHAPTER 1

INTRODUCTION

Fatigued pig syndrome refers to non-injured market hogs who arrive at the slaughterhouse nonambulatory (Ritter et al., 2005). Fatigued pigs are one subgroup of total transport losses in the swine industry, which include dead and injured pigs as well, and were estimated to cost the swine industry between $50 and $100 million per year (Ellis et al., 2003). This syndrome can be caused by antemortem stress, as Anderson et al. (2002) reported nonambulatory pigs displayed greater signs of acute stress than ambulatory pigs in the yard of a packing plant. As the swine industry placed more importance on leaner, more muscular carcasses, the incidence of stress and anxiety within market hog genetic lines increased (Shea-Moore, 1998), increasing the risk of pale, soft, and exudative (**PSE**) meat (Warris, 2000). While PSE is associated with multiple quality defects, the most easily noticed is the much paler color of the fresh meat (Warris, 2000).

Historically, meat color was considered the most important factor consumers used when making purchasing decisions (Dunsing, 1959; Faustman and Cassens, 1989). More recent studies indicated color is one of the top three attributes (Vierck et al., 2018), and a study in pork specifically determined that price was picked as the top attribute by the most consumers, with color following only 2% behind (Rice et al., 2019). Because consumers place such importance on this trait, it can have large economic impacts. Recently, it was reported that discoloration in the beef industry results in economic losses of approximately $3.73 billion annually (Ramanathan et al., 2022). Other beef studies

report that even slight discoloration can result in a decreased willingness to pay of $2.00/lb, or 50% of product price, respectively (Grebitus et al., 2013; Feuz et al., 2020). While these estimates do not equate to pork, discoloration certainly results in economic losses to the pork industry as well. Surface discoloration in meat products is caused by the accumulation of metmyoglobin (**MMb**), which is associated with brown color rather than either of the other two common forms of myoglobin, deoxymyoglobin (**DMb**) that is purple, or oxymyoglobin (**OMb**) that is associated with the bright red color of fresh red meat (Faustman and Phillips, 2001).

The ability of fresh meat products to reduce MMb is critical to ensuring greater color stability in retail display situations (Mancini et al., 2004). Studies by Arihara et al. (1989) and Bekhit et al. (2003) demonstrated the importance of NADH-dependent pathways on metmyoglobin reducing activity (**MRA**). Increasing the pool of NAD+, which is converted to NADH (Sammel et al., 2002), may enhance MRA in postmortem muscle.

Nicotinamide riboside (**NR**) is a naturally occurring vitamin B3 analog (Chi and Suave, 2013). Multiple studies demonstrated the ability of NR to increase NAD+ concentration in skeletal muscle in both mice and humans (Canto et al., 2012; Khan et al., 2014; Martens et al., 2018). In several studies, increased muscle NAD+ concentrations was associated with increased mitochondrial content, mitochondrial functionality, oxygen consumption, and ATP production in both healthy and diseased mice models (Khan et al., 2014; Ryu et al., 2016; Zhang et al., 2016). In meat products, mitochondrial activity prevents myoglobin pigment oxygenation from occurring as quickly, resulting in a darker product (Cornforth and Egbert, 1985), and presumably prolonging color stability. A

darker initial color in pork could be beneficial especially in the pork export market (Wiegand et al., 2002).

While recent studies have demonstrated NR's positive effect on muscle fiber development in avian species (Gonzalez and Jackson, 2020; Xu et al., 2021), no work has been done to explore the effects of NR in swine, with the exception of a preliminary study by Gonzalez et al. (2019) that demonstrated increased redness and decreased chop discoloration associated with NR supplementation. Considering the positive effects of NR on skeletal muscle and the results of the pilot study, the hypothesis of this experiment was that oral supplementation of NR to market hogs would improve pork color by increasing redness, and increase color stability. Therefore, the objective of this study was to determine the effects of NR supplementation on the color and color stability of pork loin chops.

CHAPTER 2

REVIEW OF LITERATURE

Stress Effects on Meat Quality

Antemortem stress in production livestock can be caused by many different factors. Psychological factors include adverse handling techniques, fear, and the introduction to new social hierarchies while physical factors can include temperature stress, injury, and exercise, all of which can lead to depletion of energy storage (Schaefer et al., 2001). Stress, or how the body responds to physical of psychological strain, has become an important topic of research in production animal industries as it directly impacts animal welfare, product quality, and consumer preferences. Very few recent studies address consumer perception of animal products as widely as Spain et al. (2018) who found that 78% of consumers in the United States are at least somewhat concerned about the welfare of animals providing their food products, with 74% of respondents admitting they pay closer attention to welfare labels than they did five years ago, indicating that it is of increasing importance to consumers to reduce porcine antemortem stress. Reducing stress can also have an economic impact by improving meat quality and satisfying consumer demands so they will be willing to pay more for a product. Spain et al. (2018) reported that consumers responded to the survey that they were willing to pay an average of $0.98 per pound more for a poultry breast with a welfare guarantee, which was a 48% increase in price for this product. Consumers' trust in various sectors of animal agriculture was also gauged in Spain's study, where the pork industry scored the

lowest percentage of trust (45%) out of dairy, egg, beef, and poultry. Porcine stress can cause meat quality defects. The most common meat quality defect caused by stress in pigs is Pale, Soft, and Exudative (**PSE**) meat. This defect is characterized by meat that is lighter pink, softer to the touch, and exudes more water than normal meat. This is primarily due to the change in pH decline (Briskey, 1964) of meat from stressed animals as the short term stress activates glycogen stored in the muscle, which is converted to lactate in the anaerobic state after the animal dies, contributing to the pH decline. The pH decline for PSE meat can either be at a faster rate than normal meat, or continue past the normal ultimate pH of meat. This increased pH decline causes denaturation of some proteins, releasing more water from the meat, which also leeches myoglobin from the product, leading to the pale, soft, and exudative characteristics of this meat. Other theories as to the cause of increased rate of pH decline include increased Ca^{2+} being released from the sarcoplasmic reticulum (Wang et al., 2019), and increased muscle ATPase activity (Scopes, 1974), but all theories include increased rate of postmortem glycolysis.

Handling can be a source of stress that causes pH decline issues. Gilts and boars subjected to an electric prod one minute prior to slaughter both exhibited a decreased pH in the *longissimus lumborum* and *semimembranosus* 45 minutes postmortem when compared to gilts and boars moved gently with a board (Van der Wal et al., 2019) with the stressed gilts having an even lower pH than the stressed boars. Another study compared the use of electrical goad to snare and non-stressed pigs (Kuchenmeister et al., 2005). The pH of the *longissimus lumborum* 45 minutes postmortem was lower for pigs treated with the electrical prod than control or snared pigs. Heart rate was measured while

treatment was being applied prior to slaughter as heart rate can be an indicator of stress. The heart rate of the gently moved pigs did increase to an average of 175 beats per minute (**BPM**) while they were being moved, and those treated with the electrical prod increased to an average of 200 BPM. Pigs subjected to the snare prior to slaughter actually had an average heart rate closer to 100 BPM, indicating this was not an effective stressor which may explain the lack of difference in pH and other measurements between snare and control pigs. Supporting the pH decline data, Kuchenmeister et al. (2005) also found an increased amount of lactate in the muscle of the pigs subjected to electrical prod when compared to the control and snared pigs.

The risk of PSE can be affected by transportation prior to slaughter as well. Transportation time and stocking density are two factors that can affect stress levels. Guàrdia et al. (2014) studied the effects of these two factors on the risk of PSE. At the shortest transportation time (1 hour), the lower stocking densities (0.5 m^2/100 kg pig) caused increased percentage of PSE risk, approximately 4% increase from highest to lowest density. As transportation time increased, however, the trend reversed. The longest trip (7 hours) had the greatest percent risk of PSE in trips with the most dense stocking rate (0.25 m^2/100 kg pig), approximately 2% different from the lowest risk combination. This indicates that ideal stocking density changes based on trip length, and both of these factors can cause increased stress in pigs. For trips approximately 3 hours in length, the risk of PSE was the same for all stocking densities.

Lairage time can also effect stress in pre-slaughter pigs. Lairage can either be a source of additional stress for a pig due to unfamiliar settings and social hierarchies, lack of food, or it can be additional time for a pig to recover from the stress of transport. A

study classified lairage times at a slaughter plant into short (8 minutes to 2.7 hours) and long (14 to 21 hours) to determine the effect of lairage time on meat quality (Dokmanovic et al., 2014). In this classification, a shorter lairage time had a detrimental effect on drip loss, meat color, and percentage of PSE carcasses. Pigs subjected to longer lairage times had less drip loss by 1%. Pork chops from pigs subjected to longer lairage times also had a decreased CIE L* value, indicating a darker product, and a greater sensory color score indicating a darker, more red product as well. The authors of this study also reported a 52% decrease in PSE carcasses in the long lairage group when compared to the short lairage group.

Dietary Intervention

Countermeasures to antemortem stress and fatigue come in the form of handling guidelines and dietary intervention strategies. Certain dietary interventions have been successful in mitigating the effects of stress. Magnesium increased both the 0 minute and 45 minute *longissimus lumborum* pH of 3 hour-transportation stressed pigs when compared to pigs that had no stress, magnesium supplement with no stress, and transportation stress with no supplement (Apple et al., 2005). The loins of pigs supplemented with Vitamin E and Vitamin C had increased Japanese color scores when compared to pigs fed conventional diets (Peeters et al., 2006). A greater Japanese color score indicates a darker red color that is more desirable in both domestic and export markets. While vitamin E-supplemented pigs had a slight yellow tint to their fat, the vitamin C supplement was promising as it also decreased a* value, which is an objective measure of redness.

Meat Color

Meat color has historically been considered one of the most important factors that consumers consider when purchasing a meat product in the grocery store (Issanchou, 1996). Recently, certain beef studies noted that color was consistently in the top three attributes of consideration for consumers when choosing a product (Wilfong et al., 2016a; Wilfong et al., 2016b; Vierck et al., 2018; Rice, 2019). Color being such an important factor in the purchasing decision makes it a meat quality trait of great economic importance. Smith et al. (2000) estimated losses of $1 billion annually to the meat industry due to surface discoloration of products, but as time progressed, economic impact of discoloration has increased. Ramanathan et al. (2022) estimated losses of $3.73 billion annually due to premature discoloration to the beef industry alone. Other beef studies indicated a consumers' willingness to pay decreased significantly by $2.00/lb or 50% of product price, respectively (Grebitus et al., 2013; Feuz et al., 2020). While these studies were conducted on beef, color is still a primary trait of concern to the consumer, and discoloration can result in large economic losses to the pork industry as well.

Pale, soft, and exudative pork is often considered a color defect as the first indicator of the issue is the color (Warris et al., 2000). The PSE pork is associated with a light color, and therefore L* value on the CIE scale is potentially the best indicator of the pinkness of pork (Brewer et al., 2001). The reason for this pale pink color in PSE meat is that a low pH denatures proteins allowing for myoglobin in the water of meat to leak out, reduces electrostatic repulsion within muscle fibers which increases the refractive index (Swatland et al., 2008). An increased refractive index will increases surface reflectance

on a meat product, and increase scattering from light penetrating myofibrils (Swatland et al., 2008).

Additionally, a low pH can create a positive environment for myoglobin oxidation (Aditzey and Nurul, 2011). Swine that experience acute pre-slaughter stress are susceptible to this defect and controlling the rapid pH decline caused by stress that causes PSE can produce a pork product with a more desirable color (Scheffler and Gerrard, 2007).

The protein primarily responsible for maintaining meat color is known as myoglobin (Livingston and Brown, 1981). Three different forms of myoglobin are associated with different red meat colors (Brooks, 1935). Surface discoloration of meat products is attributed to the accumulation of the ferric myoglobin, called metmyoglobin which occurs when myoglobin goes through an oxidation reaction (Wallace et al., 1982). In order to turn metmyoglobin to another form of myoglobin that is not considered discolored, the myoglobin molecule must be chemically reduced. Deoxymyoglobin, which gives a more purple color to the meat, and oxymyoglobin, the oxygenated form of myoglobin are the other naturally occurring forms of myoglobin (Brooks, 1935). Oxymyoglobin is what is commonly associated with bright, fresh colors of red meat (Wallace et al., 1982).

The reducing ability, called metmyoglobin reducing ability (**MRA**) of a muscle is considered to be an indicator of how color stable that muscle will be (Reddy and Carpenter, 1991). Oxygen consumption rate (**OCR**) can also be related to the color stability of a meat product, as this is thought to be an indication of mitochondrial oxygen consumption that occurs in postmortem muscle (Bendall and Taylor, 1972; Madhavi and

Carpenter, 1993). The decline of mitochondrial function as meat ages is associated with an increase in OCR due to oxygen permeating into the muscle instead of being metabolized by postmortem mitochondria (Bendall and Taylor, 1972).

Although some research groups indicate that NADH may play a large role in reducing metmyoglobin and promoting color stability (Bekhit et al., 2003), little research has been done in this area. Sammel et al. (2002) indicated that one of the least color stable areas of the beef *semimembranosus* muscle also had smaller amounts of NAD+ than other areas. This area of the muscle also had lower MRA and OCR, indicating that NAD+ or NADH amounts may affect MRA and OCR of meat products. An in vitro study of beef muscle extracts suggested that an NADH-dependent reducing system can reduce metmyoglobin (Osborn et al., 2003). One group proposed a pathway in which lactate dehydrogenase in enhanced beef increased NADH and thereby reducing ability, leading to more color stable beef (Mancini et al., 2004).

Nicotinamide Riboside

Nicotinamide riboside (**NR**) is a vitamin B3 analog compound that can be found in very low amounts in yeast, bacteria, and milk products (Chi and Suave, 2013). As a precursor for nicotinamide adenine dinucleotide (**NAD+**), NR synthesized in laboratories increased NAD+ levels in both mice and humans (Khan et al., 2014; Martens et al., 2018). Oral supplementation of NR to mice increased NAD+ content in skeletal muscle (Canto et al., 2012), and in humans as well (Martens et al., 2018). The increased level NAD+ in skeletal muscle due to NR supplementation is associated with increased mitochondrial content as measured by mitochondrial DNA concentration, mitochondrial protein concentration, and increased oxygen consumption in mice (Canto et al., 2012;

Khan et al., 2014; Ryu et al., 2016). Improved mitochondrial function can be attributed to NR supplementation as well due to increased mitochondrial cristae (Canto et al., 2012; Khan et al., 2014).

The supplementation of NR also positively affects adenosine triphosphate (**ATP**) production (Khan et al., 2014; Ryu et al., 2016; Zhang et al., 2016) as would be expected due to the increased number and function of mitochondria in supplemented animals. The combination of the effects that NR has on NAD+ levels, ATP levels, and mitochondrial numbers and function reveals the potential of this supplement to have numerous positive effects on skeletal muscle fibers in multiple species. Recent studies have shown the positive effect that NR can have on poultry myogenesis (Gonzalez and Jackson, 2020; Xu et al., 2021). Other studies demonstrated the ability of NR to increase muscle function: by increasing ATP and oxygen consumption, NR has improved endurance in mature mice tested on treadmills (Zhang et al., 2016), as well as enhanced grip strength in mice with muscular dystrophy (Ryu et al., 2016).

CHAPTER 3

EFFECT OF VITAMIN B3 DOSE ON FATIGUED BARROW CARCASS CHARACTERISTICS AND LOIN CHOP COLOR STABILITY[1]

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[1] Gravely, M.E., H.M. Alcocer, S.R. Brannen, D.A. Alambarrio, J.J. McDonald, S.A. Devane, D.B. Davis, O. Ellis, X. Xu, A.M. Stelzleni, and J.M. Gonzalez. To be submitted to *Journal of Animal Science.*

ABSTRACT

The objective of this study was to determine the effects of nicotinamide riboside (**NR**) supplementation on fresh pork color, color stability, yield, and other composition measures. One hundred barrows were supplemented different levels of NR [0 mg•hd^{-1}•kg body weight^{-1} (**CON**), 15 mg•hd^{-1}•kg body weight^{-1} in feed (**15NR**), 30 mg•hd^{-1}•kg body weight^{-1} in feed (**30NR**), 45 mg•hd^{-1}•kg body weight^{-1} in feed (**45NR**), and 45 mg•hd^{-1}•kg body weight^{-1} in an oral drench (**DRE**)] 11 d prior to a fatigue-inducing event and then harvested. Slaughter and fabrication yields, and pH of *semitendinosus*, *biceps femoris*, *tensor fascia latae*, and *longissimus* were collected. Loins were aged 10 d in vacuum bags, then chops were cut and used for an 8-d simulated retail display, metmyoglobin reducing ability, oxygen consumption, water holding capacity, Warner-Bratzler shear force, histology, and proximate composition. There were no day of display (**DOD**) × treatment (**TRT**) interactions or hour (**HR**) × treatment (**TRT**) interactions for any variables ($P \geq 0.15$), except for pH decline of the *tensor fascia latae* pH ($P = 0.04$). Postmortem pH was measured at 1, 2, 3, 4, 5, 6, 12, and 24 h. In the first h postmortem, 45NR had decreased ($P \leq 0.02$) pH from the other treatments, while in h 2, DRE pH was decreased from CON and 15NR ($P < 0.01$), but not 30NR or 45NR, which did not differ from each other or any other treatments ($P \geq 0.07$). No differences between treatment occurred for any other hours ($P \geq 0.07$). No TRT effects were observed for any variables ($P \geq 0.15$). There was a DOD effect on subjective color and discoloration score (on a 100-point sliding line scale), L*, a*, b* values, MRA, and OC ($P < 0.01$). Subjective color score was greatest on d-0 ($P \leq 0.01$). Day-1 and -2 score did not differ ($P = 0.19$), but were greater than remaining days ($P \leq 0.01$). Score on d-3 was greater than remaining

days ($P \leq 0.05$). Day-4 and -5 score did not differ ($P = 0.17$), but d-4 was greater than d-6, -7, and -8 ($P \leq 0.03$). Score on d-5 and -6 were not different ($P = 0.43$), but were greater than d-7 and -8 ($P \leq 0.01$), which were not different from one another ($P = 0.34$). Discoloration on d-0, -1, and -2 were not different ($P \geq 0.18$), but d-0 discoloration was less than remaining days ($P \leq 0.01$). Discoloration on d-1, -2, and -3 did not differ ($P \geq 0.19$), but were all less than remaining days ($P \leq 0.01$). Day-5 discoloration was greater ($P < 0.01$) than d-4, and each subsequent day had increased discoloration from all previous days through d-8 ($P \leq 0.01$). Considering the results of this study, NR supplementation was unable to improve pork color stability.

INTRODUCTION

Fatigued pig syndrome refers to non-injured market hogs who arrive at the slaughterhouse nonambulatory (Ritter et al., 2005). Fatigued pigs are one subgroup of total transport losses in the swine industry, which include dead and injured pigs as well, and were estimated to cost the swine industry between $50 and $100 million per year (Ellis et al., 2003). This syndrome can be caused by antemortem stress, as Anderson et al. (2002) reported nonambulatory pigs displayed greater signs of acute stress than ambulatory pigs in the yard of a packing plant. As the swine industry placed more importance on leaner, more muscular carcasses, the incidence of observed stress responses and nervous behavior within market hog genetic lines increased (Shea-Moore, 1998), increasing the risk of pale, soft, and exudative (**PSE**) meat (Warris, 2000). While PSE is associated with multiple quality defects, the most notable is the paler color of the fresh meat (Warris, 2000).

Historically, meat color was considered the most important factor consumers used when making purchasing decisions (Dunsing, 1959; Cassens et al., 1989). More recent studies indicated color is one of the top three attributes (Vierck et al., 2018), and a study in pork specifically determined that price was picked as the top attribute by the most consumers, with color following only 2% behind (Rice et al., 2019). Because consumers place such importance on this trait, it can have large economic impacts. Recently, it was reported that discoloration in the beef industry results in economic losses of approximately $3.73 billion annually (Ramanathan et al., 2022). Other beef studies report that even slight discoloration can result in a decreased willingness to pay of $2.00/lb, or 50% of product price, respectively (Grebitus et al., 2013; Feuz et al., 2020). While these estimates do not equate to pork, discoloration certainly results in economic losses to the pork industry as well. Surface discoloration in meat products is caused by the accumulation of metmyoglobin (**MMb**), which is associated with brown color rather than either of the other two common forms of myoglobin, deoxymyoglobin (**DMb**) that is purple, or oxymyoglobin (**OMb**) that is associated with the bright red color of fresh red meat (Faustman and Phillips, 2001).

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While recent studies have demonstrated NR's positive effect on muscle fiber development in avian species (Gonzalez and Jackson, 2020; Xu et al., 2021), no work has been done to explore the effects of NR in swine, with the exception of a preliminary study by Gonzalez et al. (2019) that demonstrated increased redness and decreased chop discoloration associated with NR supplementation. Considering the positive effects of NR on skeletal muscle and the results of the pilot study, the hypothesis of this experiment was that oral supplementation of NR to market hogs would improve pork color and increase color stability. Therefore, the objective of this study was to determine the effects of NR supplementation on the color and color stability of fresh pork.

MATERIALS AND METHODS

Experimental procedures were approved by the University of Georgia (UGA) Intuitional Animal Care and Use Committee (AUP # A2020 03-004-A8).

Live Animal Management

One hundred finishing barrows (CG36 × P26; Choice Genetics, West Des Moines, IA) were transported 0.05 km to the University of Georgia Large Animal Research Unit (**LARU**; Athens, GA). Barrows were managed as described in Alcocer et al. (2022). Briefly, barrows were housed individually in 5 × 1.5 m pens in an environmentally controlled room in the LARU prior to supplementation of NR for an acclimation period in each repetition for 25 ± 11 d. Each pen contained a 2-hole dry feeder (Farmwield, Teatopolis, IL) and nipple waterers, allowing *ad libitum* access to both feed and water. In repetitions 1 through 3, barrows were weighed 12 d prior to the first harvest, and randomly allocated to 2 separate harvest groups. The first harvest group began supplementation 11 d prior to harvest, while the second group began supplementation 4 days later. Within each harvest group, barrows were ordered based on body weight and randomly assigned to a treatment group. In repetitions 4 and 5, barrows were classified into light and heavy groups prior to arrival at the LARU. The heavy group arrived at LARU 7 d prior to the light group. Barrows in each group were ordered by weight, and randomly allocated to a treatment group. Heavy groups began supplementation a week prior to light groups, resulting in heavy groups being harvested a week prior to light groups.

All barrows were offered a commercial finishing diet mix as reported in Alcocer et al. (2022), and were allocated to one of five treatments with the following amounts of NR (Chromadex; Los Angeles, CA) supplemented beginning 11 d prior to a fatigue-inducing event and harvest: 0 mg•hd^{-1}•kg body weight^{-1} (**CON**), 15 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (**15NR**), 30 mg•hd^{-1}•kg body weight^{-1} mixed in with feed

(**30NR**), 45 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (**45NR**), and 45 mg•hd^{-1}•kg body weight^{-1} provided in an oral drench form (**DRE**). In repetitions 1 through 3, the oral drench was provided in Karo Syrup (ACH Food Companies, Oakbrook Terrace, IL) in a syringe, while in repetitions 4 and 5, the drench was provided in raw cookie dough (Nestle; Arlington, VA). Dosages from d-0 to d-5 of supplementation were calculated based on average daily intake (**ADI**) and body weight prior to supplementation, and dosage for d-5 to d-10 of supplementation were based on ADI from d-0 to d-5 of supplementation, and body weight from d-5.

On the day of each harvest, barrows in the group being harvested were subjected to a performance test as described in Alcocer et al. (2022). Repetitions 1 through 3 were completed with 20 barrows each; repetitions 4 and 5 were completed with 19 barrows each, due to one barrow in each of the last two repetitions dying from causes unrelated to the experimental procedures.

Harvest

Immediately after the performance test, barrows were individually transported via truck and trailer 0.05 km to the University of Georgia Meat Science and Technology Center (**MSTC**; Athens, GA) for harvest following federal-inspection approved procedures commonly utilized at the MSTC. Approximately 1 g of the *longissimus lumborum* was immediately collected following exsanguination and approximately 45 min later, another 1 g sample was collected along with a cardiac sample for mitochondrial analysis at Colorado State University (data not shown). Carcass pH was collected at 1 2, 3, 4, 5, 6, 12, and 24 h at three different locations corresponding to electromyography placement locations (*semitendinosus, biceps femoris, tensor fascia*

latae) as described by Alcocer et al. (2022) and the *longissimus lumborum* using a calibrated Hanna meat pH meter (HI99163; Hanna Instruments Inc., Woonsocket, RI). Carcasses were chilled for 24 h prior carcass evaluation and fabrication.

Carcass Fabrication

Carcass right sides were ribbed between the 10^{th} and 11^{th} ribs and loin eyes were allowed to bloom for 30 min prior to back fat and loin eye color evaluation. Back fat thickness was measured at the first rib, three-quarters of the longitudinal distance from the back bone to most distal end of the loin eye at the 10^{th} rib, and last rib by one trained researcher using a back fat probe (USDA). Loin eye areas were traced and measured using a pork loin grid (Iowa State University Extension, Ames, IA). Loin eye color, marbling, and fat color was measured by taking the average scores of three trained University personnel using the National Pork Producers Council color and marbling standards (NPPC, 2000), and Japanese Color Standards for lean color and fat color (Nakai et al., 1975). Loin eye surface objective color measurements [L*, a*, b* Commision Internationale de l'Eclairage values (**CIE**, 1976)] were measured using a HunterLab MiniScan EZ handheld spectrophotometer (Illuminant A, 10° observer, 2.54-cm apterture). Three scans were taken, and averaged for each loin eye.

Carcass left sides were weighed, fabricated into primals and subprimals by trained university researchers as designated by Institutional Meat Purchase Specifications (IMPS 402, 405, 406, 409, 413, 415, 416, 418, 420, 420A, 422; USDA, 2014) and weighed for cutability data. Whole boneless loins were vacuum packaged (3 mil; O_2 transmission rate = 50 to 70 $cm^3 \bullet m^{-2} \bullet 24\ h^{-1}$; UltraSource LLC, Kansas City, MO) and aged at 2°C for 10 d in boxes with no light penetrating packages.

Loin Cutting

After aging, loins were halved immediately posterior to the *spinalis dorsi*. Immediately posterior to that cut, eight 2.54-cm chops were cut and trimmed of any external fat. Chop 1 was designated for immunohistochemistry analyses. Chop 2 and 3 were halved into two 1.27-cm chops and used for either d-0 and d-4 of simulated retail display MRA or oxygen consumption (**OC**). Chop 4 was used for simulated retail display objective and subjective color stability analysis, and used for d-8 OC following the conclusion of the display period. Chop 5 was used for d-8 MRA analysis. Chop 6 was vacuum sealed and stored at -20°C for measurement of Warner-Bratzler Shear Force (**WBSF**), and chops 7 and 8 were vacuum sealed and frozen at -20°C for potential use in further studies.

Anterior to where the loin was halved, four 2.54-cm chops were cut and trimmed of external fat. Chop 9 (most anterior of this section) was used for drip loss analysis, chop 10 was used for expressible moisture analysis, and chop 11 was designated for water binding analysis on d-0 of the simulated retail display. Chop 12 was vacuum sealed and stored at -20°C for proximate composition analysis.

Simulated Retail Display

Display chops and chops designated for MRA and OC were placed on 1S Styrofoam trays (CKF 87801; CKF Inc., Langley, British Columbia, Canada) on top of an absorbent pad (Dri-Loc AC-40; Cryovac LLC, Elmwood Park, NJ) and overwrapped with polyvinylchloride film (O_2 transmission rate= 23,250 $mL \cdot m^{-2} \cdot 24\ h^{-1}$, 72 gauge; Pro Pack Group, Oakland, NJ), and placed in a coffin style retail display case (M1X-E; Hussmann, Bridgeton, MO) at 3.7 ± 0.8°C for 8 d under constant fluorescent lighting

(1,780 lux; Octron ECO; 30000 K; Sylvania Company, Versailles, KY). Chops were rotated daily front-to-back and side-to-side to account for temperature variation in the case, and case temperature was monitored using temperature loggers (Omega Engineering Inc., Norwalk, CT). Daily, objective measurements were collected using a polyvinyl-chloride-film calibrated spectrophotometer as described above. Spectral data (400 to 700 nm) was used to calculate surface oxymyoglobin, deoxymyoglobin, and metmyoglobin percentages using the equations of Krzywicki (1979) and chroma and hue angle were calculated as described in the American Meat Science Association (**AMSA**) Meat Color Measurement Guidelines (AMSA, 2012).

Subjective color scores were collected daily following the visual evaluation methods of Gonzalez et al. (2020) with minor modifications. Briefly, data was collected using Qualtrics survey software on iPads (Apple Inc., Cupertino, California) from panelists oriented to pork discoloration patterns. The lean color scale was evaluated on a continuous line scale from 1 to 100 with anchor points at the minimum, maximum, and the middle, where a score of 1 = NPPC 1, 20 = NPPC 2, 40 = NPPC 3, 60 = NPPC 4, 80 = NPPC 5, and 100 = NPPC 6. Discoloration was also evaluated on a sliding scale, this time from 0 to 100, where anchors were 0 = 0% discoloration, 50 = 50% discoloration, and 100 = 100% discoloration. Panelists' scores were averaged each day.

Metmyoglobin Reducing Activity and Oxygen Consumption

Procedures from Gonzalez et al. (2009) were followed with modifications. Chops were placed in 500 mL glass beakers with 400 mL of 0.3% sodium nitrite solution at 25 $\pm$ 2°C for 20 min with the display surface facing up. Chops were removed from solution, excess solution was blotted off, chops were immediately vacuum packaged, and surface

spectral reflectance data was collected as described above. Packaged chops were incubated at 30°C, and subsequent spectral data were collected at 1 and 2 h. Spectral reflectance data were used to calculate percent surface metmyoglobin as described above and MRA was calculated as percent reduction in surface metmyoglobin from 0 to 2 h as published in the AMSA Meat Color Measurement Guidelines (AMSA, 2012).

Oxygen consumption analysis was performed according to Ramanathan et al. (2019) with modifications. Chops designated for d-0 OC were allowed to bloom for 30 min prior to analysis, and chops allocated to d-4 and d-8 OC were not subjected to blooming after removal from the retail case. Chops were removed from packaging and vacuum packaged, and spectral data were collected and processed as described previously. After 40 min, chops were rescanned and spectral values from 0 min and 40 min were used to calculate oxymyoglobin and oxygen consumption was calculated as percent change in oxymyoglobin from initial to final as reported in the AMSA Meat Color Measurement Guidelines (AMSA, 2012).

Water Holding Capacity

Three assays were used to measure water holding ability. Chops allocated to drip loss were weighed, hung on a fish hook inside a Whirl-Pak bag (B01445; Nasco Whirl-Pak, Madison, WI) at 4°C for 24 h so the meat was not touching the bag, and reweighed. Percent drip loss was calculated as the [(initial weight – final weight)/(initial weight) × 100].

The methods of Phelps et al. (2015) were followed for measurement of expressible moisture (**EM**) and water binding ability (**WBA**). Briefly, expressible moisture was measured by placing one 5 g sample of loin on top of 25 g of 4-mm glass

beads inside a 50 mL conical tube. Conical tubes with sample were centrifuged at 900×*g* for 10 min. Muscle samples were reweighed and percent expressible moisture was calculated as [(initial weight – centrifuged weight)/(initial weight) × 100].

Water binding ability was analyzed by blending 100 g of muscle with 300 mL of distilled water in a Waring blender (Waring Products Division, Hartford, CT). After blending, two 35 g samples were transferred to separate 50 mL conical tubes and centrifuged at 900×*g* for 10 min. Supernatant was measured volumetrically, averaged for each chop, and swelling percent was calculated as [300 – (11.43 × supernatant volume).

Warner-Bratzler Shear Force

Cooking and WBSF were performed according to AMSA Research Guidelines for Cookery and Evaluation (AMSA, 2016). Frozen chops were weighed, thawed on top of absorbent pads on trays covered with polyvinylchloride film at 2°C for 24 h, and reweighed prior to being cooked on a Cuisinart Griddler Elite (Model GR-300WSP1; Cuisinart, Stamford, CT) set at 232°C. Internal temperature of each chop was measured using a ThermoPro Meat Thermometer (ThermoPro-TP-19H; Toronto, Canada). Chops were pulled from the griddle at 64°C and peak temperature averaged 74 ± 3°C. Chops were then reweighed and allowed to cool on a tray with absorbent pads under polyvinylchloride film for 24 h at 4°C. A minimum of two 1.27-cm cores were taken from each chop, parallel to muscle fiber orientation. Cores were sheared perpendicular to the muscle fibers using an Instron Universal Testing Machine (Instron Dual Column Model 3365; Instron Corp., Norwood, MA) equipped with a Warner-Bratzler shear head, 51 kgf load cell, using a crosshead speed of 25 cm/min. Bluehill software (Instron Corp.)

recorded peak shear force in kgf for each core, and all cores from a chop were averaged to obtain average peak force per chop.

Proximate Analysis

Chops allocated to proximate composition analysis were thawed for 24 h at 4°C, minced, and frozen in liquid nitrogen before being powder homogenized using a Waring blender (Waring Commercial, Stamford, CT). Duplicate 1 ± 0.1 g homogenized samples were placed into Ankom filter bags (ANKOM Technology; Macedon, NY). Sealed filter bags were placed into individual aluminum pans and weight of the pan, bag, and sample was recorded. Pans were placed into an oven at 100°C for 12 h, cooled in a desiccator for 15 min, and reweighed to determine percent moisture as [(initial weight – dried weight)/initial weight × 100]. These samples were then placed into an Ankom petroleum ether fat extractor (Model XT-15; ANKOM Technology) for 60 min at 90°C. Following fat extraction, samples were dried in a 100°C oven to remove residual ether, cooled in the desiccator for 15 min, and reweighed. Lipid percent was calculated as [(dried weight – extracted weight)/initial weight × 100].

Triplicate powder homogenized samples weighing 0.2 g were weighed into aluminum foil. Nitrogen was measured by combusting samples in a Leco Nitrogen Analyzer (Model FP268; Leco Corporation, St. Joseph, MI). The equation used for calculating crude protein was (% nitrogen × 6.25).

Immunohistochemistry

A 1×1×1-cm muscle tissue section was taken from the medial section of each chop and embedded in optimal cutting temperature tissue freezing media (Neg-50; Epredia, Kalamazoo, MI) with muscle fibers running perpendicular to the bottom of the

freezing mold. Tissue was frozen using liquid-nitrogen cooled 2-methylbutane, and stored at -80°C until used for cross sectioning. Two slides with 8-µm cryosections were obtained from each section on positively charged slides (Cardinal Health, Waukegan, IL). One slide was used for fiber typing and the other for succinate dehydrogenase (**SDH**) staining.

Fiber typing was performed according to Paulk et al. (2014) with minor modifications. Briefly, cryosections were incubated to block non-specific binding sites with a blocking solution of 5% horse serum and 0.2% TritonX-100 in phosphate buffered saline (**PBS**) for 30 min. Cryosections were then incubated with a primary antibody solution containing blocking solution, 1:10 supernatant myosin heavy chain, type I, IgG2b (BA-D5; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), 1:10 supernatant myosin heavy chain, type IIA, IgG1 (SC-71; Developmental Studies Hybridoma Bank), and 1:100 supernatant myosin heavy chain, type IIB, IgM (BF-F3; Developmental Studies Hybridoma Bank) at 4°C overnight. Primary antibody solution was removed and sections were washed 3 times for 5 min with PBS. Cryosections were then incubated for 45 min with a secondary antibody solution containing blocking solution, 1:1,000 Alexa Fluor 633 goat anti-mouse IgG2b (Invitrogen, San Diego, CA) for BA-D5, 1:1,000 Alexa Fluor 488 goat anti-mouse IgM (Invitrogen) for BF-F3, 1:1,000 Alexa Fluor 594 goat anti-mouse IgG1 (Invitrogen) for SC-71, and 1:1,000 WGA Alexa Fluor 594 (Invitrogen). Sections were washed again 3 times for 5 min using PBS and coverslipped with 5 µL of 9:1 glycerol for imaging.

The methods of Noel et al. (2015) were followed for SDH staining. Slides for SDH staining were incubated at 37°C for 45 min in a solution containing 50% nitro blue

tetrazolium solution (1% nitro blue tetrazolium in Milli-Q water), 25% phosphate buffer solution (20% potassium phosphate monohydrate, 11% disodium hydrogen phosphate in Milli-Q water), and 25% sodium succinate solution (2.7% sodium succinate dibasic hexahydrate in Milli-Q water). Slides were then washed in Milli-Q water 3 times for 1 min each, and coverslipped with 5 μL of 9:1 glycerol for imaging.

Cryosections were imaged on an Echo Revolve D-319 microscope (Echo, San Diego, CA). Four photomicrographs from fiber typing cryosections were imaged at 100× magnification, while four SDH photomicrographs were captured at 40× magnification at corresponding locations on the sample. A minimum of 1,000 fibers per sample were analyzed for fiber type distribution and cross-sectional area (**CSA**) using Echo Revolve software v6.4 (Echo), and a minimum of 1,000 fibers per sample were analyzed for SDH staining using ImageJ software (National Institutes of Health, Bethesda, MD). Fibers positive for BA-D5 were considered type I fibers, those positive for SC-71 were type IIA, those positive for BF-F3 were IIB, and those that stained weakly for both SC-71 and BF-F3 were considered type IIX fibers. The SDH intensity was measured using Image J software (NIH), where a value of 0 indicated black (most intense) and 100 indicated white (least intense).

Statistical Analysis

Carcass, proximate composition, and WBSF data were analyzed as randomized complete block design with carcass as the experimental unit. Treatment served as the fixed effect and kill block served as the random effect. Muscle fiber morphometric and SDH data were analyzed with the same model. Color-stability and pH data were analyzed as a randomized complete block design with repeated measures. The pH data were sorted

by muscle and analyzed independently. Treatment, Time, and their interaction served as fixed effects, while block served as the random effect. Time served as the repeated measure with barrow as the subject and compound symmetry as the covariance structure. Metmyoglobin reducing ability and OC data were analyzed as a randomized complete block design with a 5 × 3 factorial arrangement. Treatment, Time, and their interactions served as fixed effects and kill block served as the random effect. Time served as the repeated measure with barrow as the subject and compound symmetry as the covariance structure. Differences were considered significant at $P \leq 0.05$.

RESULTS

Carcass, pH, and Cutout Measurements

Treatment had no effect on live weight, hot carcass weight, cold side weight, dressing percentage, or shrink percentage ($P \geq 0.36$; Table 1). There were no TRT × HR interactions or TRT effects for *semitendinosus*, *biceps femoris*, or *longissimus lumborum* pH ($P \geq 0.15$; Fig. 1); however, there was an HR effect for these muscles ($P < 0.01$). In the *semitendinosus*, hour 1 pH was greatest ($P < 0.01$). Hour 2 pH was greater than remaining hours ($P \leq 0.02$), but was not different from hour 3 ($P = 0.34$). Hour 3 pH was not different from pH at hours 4 or 5 ($P \geq 0.15$), but was greater than pH at hours 6, 12, and 24 ($P \leq 0.05$). The pH at hours 4, 5, 6, 12, and 24 were not different from one another ($P \geq 0.26$). In the *biceps femoris*, hour 2 pH was decreased when compared to hour 1 pH ($P \leq 0.01$), and hour 3 pH was decreased when compared to hour 2 pH ($P \leq 0.02$). The pH at remaining hours were not different from one another ($P \geq 0.07$), but were all less than pH at hours 1, 2, and 3 ($P < 0.01$). In the *longissimus lumborum*, hour 1 had the greatest pH when compared all other timepoints ($P \leq 0.01$). Hour 2 and 3 pH did not

differ from one another ($P = 0.09$), but hour 2 pH was greater than the pH at all subsequent timepoints ($P \leq 0.01$). Hour 3 and 4 pH were not different from one another ($P = 0.15$), but hour 3 pH was greater than the following hours ($P \leq 0.01$). Hour 4 pH was greater than the pH at all remaining hours ($P \leq 0.01$), except for hour 12 pH ($P = 0.14$). The pH at hours 5, 6, 12, and 24 were not different from one another other ($P \geq 0.12$).

Analysis of the *tensor fascia latae* pH showed that there was a TRT × HR interaction ($P = 0.04$; Fig. 1). At hour 1, the pH of the *tensor fascia latae* muscle from pigs receiving the CON, 15NR, 30NR, and DRE diets did not differ from one another ($P \geq 0.09$), but the *tensor fascia latae* muscle from pigs consuming the 45NR diet had decreased muscle pH when compared to all other treatments ($P \leq 0.02$). At h 2, the pH of the *tensor fascia latae* muscle from pigs receiving the CON, 15NR, 30NR, and 45NR did not differ ($P \geq 0.24$). Pigs receiving DRE diets had decreased muscle pH compared to the pH of muscle of those consuming the CON, 30NR, and 45NR diets ($P < 0.01$), but was not different from the pH of those consuming the 15NR diets ($P \geq 0.07$). No differences between treatments were observed for the remaining hours ($P \geq 0.07$).

Treatment had no effect on first rib, tenth rib, last rib fat thickness, and loin eye area ($P \geq 0.21$; Table 2). There were no TRT effects for color attributes including NPPC color, NPPC marbling score, Japanese lean color score, Japanese subcutaneous fat color score, Japanese marbling color score, L*, a*, or b* ($P \geq 0.15$). Treatment had no effect on any carcass cutout measures ($P \geq 0.34$; Table 3).

Simulated Retail Display

There were no TRT × DOD interactions or TRT effects for subjective color score, discoloration, L*, a*, b* ($P \geq 0.23$; Fig. 2); however, day of display had an effect on all

color measurements ($P \leq 0.01$). Subjective color score was greatest on d-0 ($P \leq 0.01$). Day-1 and -2 color scores did not differ ($P = 0.19$), but were greater than all other days (P £ 0.01). Day-3 color scores were greater than remaining days ($P \leq 0.05$). Day-4 color score was not different ($P = 0.17$) from d-5 scores, but was greater than all other d ($P \leq$ 0.03). Day-5 and -6 color score were not different from one another ($P = 0.43$), but both were greater than d-7 or -8 ($P \leq 0.01$), which did not differ from one another ($P = 0.34$).

Day-0 discoloration score did not differ from d-1 or -2 ($P \geq 0.18$) but was decreased when compared to all other days ($P \leq 0.01$). Day-1, -2 and -3 discoloration were not different from one another ($P \geq 0.19$), but all were decreased in comparison to the remaining days ($P \leq 0.01$). Day-5 was increased ($P < 0.01$) in discoloration score when compared d-4, and discoloration score on each following day was increased from all preceding days ($P \leq 0.01$).

Day-0 and -1 L* values were not different ($P = 0.09$; Fig. 3), but d-0 L* values were greater than all other days ($P \leq 0.01$). Day-1 pork chop L* values were greater than all other days ($P \leq 0.01$). Day-2, -3, -4, -5, and -6 L* values did not differ from each other ($P \geq 0.42$), but d-2, -4, and -6 were greater than d-7 and -8 ($P \leq 0.02$). Day-3 L* values were not different ($P \geq 0.12$) from d-7, but were greater ($P < 0.01$) than d-8. Chops on d-8 had decreased L* values when compared to d-7 ($P < 0.01$).

The a* values on d-0 and -1 did not differ ($P = 0.07$), but were greater than a* on all other days ($P \leq 0.01$). Pork chop a* values decreased from the previous days every day from d-3 through d-7 ($P \leq 0.01$). Day-7 and -8 a* values were not different from one another ($P = 0.48$).

The b* values on d-0 were less than b* values on d-1, -2, -3, -4, -6, -7, and -8 ($P \leq 0.01$), but were not different ($P = 0.17$) from the values on d-5. Values for b* on d-2 decreased ($P < 0.01$) from those on d-1, and each following day through d-6 decreased from the previous days ($P \leq 0.01$). Chops on d-6 had greater b* ($P = 0.04$) than d-7, but were not different ($P = 0.72$) from d-8. Day-7 b* values were decreased when compared to d-8 ($P = 0.02$).

There were no TRT × DOD interactions or TRT effect for surface metmyoglobin, deoxymyoglobin, or oxymyoglobin percentage ($P \geq 0.52$; Fig. 4); however, there was a DOD effect for all ($P < 0.01$). Surface metmyoglobin increased each day from the previous days from d-0 to d-7 ($P < 0.01$), but d-6 and -7 did not differ from d-8 ($P > 0.07$). Deoxymyoglobin on d-0 was greater than all other days ($P < 0.02$). Deoxymyoglobin on d-1, -2, -3, -4, or -5 did not differ from one another ($P > 0.38$) but were all decreased when compared to d-6, -7, and -8 ($P < 0.03$). Day-6 deoxymyoglobin was less than d-7 or -8 ($P < 0.01$), and d-7 chops had less deoxymyoglobin than those on d-8 ($P < 0.01$). Oxymyoglobin was greatest on d-1 when compared to all other timepoints ($P < 0.01$). Day-0 and -2 oxymyoglobin did not differ ($P = 0.60$), but were both greater than all remaining days ($P < 0.01$). From d-2 on, each subsequent day had decreased oxymyoglobin when compared to the previous days ($P < 0.01$).

Chroma and hue angle had no TRT × DOD interactions or TRT effect ($P \geq 0.22$; Fig. 5), but DOD did affect both ($P < 0.01$). Chops on d-1 had increased ($P < 0.01$) chroma from d-0, but d-0 and d-1 both had increased chroma when compared to all other days ($P < 0.01$). Chroma decreased each day from d-2 to d-7, with each day being reduced when compared to the preceding days ($P < 0.01$). Chroma on d-7 and -8 chroma

were not different from one another ($P = 0.09$). Hue angle was smallest on d-0 ($P < 0.01$), and each day through d-7 was greater than all previous days ($P < 0.01$). Day-7 and d-8 hue angle did not differ ($P = 0.90$).

Metmyoglobin Reducing Activity and Oxygen Consumption

There were no TRT × DOD interactions or TRT effects on MRA or OC ($P \geq 0.28$; Fig. 6). Day of display had an effect on MRA and OC ($P < 0.01$). Chops on d-0 had increased MRA when compared to d-4 and -8 ($P < 0.01$). Chops on d-4 had greater ($P < 0.01$) MRA than those on d-8. Oxygen consumption in chops was increased on d-0 when compared to d-4 and -8 ($P < 0.01$), and chops on d-4 had greater ($P < 0.01$) OC than those on d-8.

Water Holding Capacity

Swelling percentage, expressible moisture percentage, and drip loss percentage were not affected by treatment ($P \geq 0.37$; Table 4).

Warner-Bratzler Shear Force

There were no treatment effects on thaw loss or cook loss percentage, cook time, final temperature, or average force ($P \geq 0.25$; Table 5).

Proximate Analysis

There was no effect of treatment on moisture, fat, or protein percentage ($P \geq 0.15$; Table 6).

Immunohistochemistry

There was no treatment effect on type I, type IIA, type IIX, or type IIB percentage, CSA, or SDH staining intensity ($P \geq 0.22$; Table 7).

DISCUSSION

Pig antemortem stress contributes to many pork quality issues, including fatigued pig syndrome and PSE (Anderson et al., 2002). Transportation has long been an area of concern in regards to antemortem stress factors, as research has consistently demonstrated transportation distance and loading density can affect stress and PSE risk (Warris et al., 1989; Gajana et al., 2013; Guárdia et al., 2014). Recently, researchers suggested other transportation factors including trailer vibration, compartment, temperature, and humidity may affect transportation stress and fatigue as well (Morris et al., 2021). Antemortem stress and the PSE condition can result in pork products' quality loss, including a plaer, less desirable surface color diminished water holding capacity (Warris, 2000). Dietary countermeasures to mitigate the effects of antemortem stress continue to be strategies of interest in mitigating the negative effects on the pork industry.

Certain dietary countermeasures have proven to have small positive effects on meat quality. Magnesium supplementation improved loin color and increased postmortem pH (Apple et al., 2000; Apple et al., 2005). Supplementation of both vitamins E and C increased subjective redness scores (Peeters et al., 2006), while feeding vitamin D_3 above nutritional requirements improved objective color measures, L* and a* (Wiegand et al., 2002). Additionally, supplementation of niacin, a vitamin B_3 compound, improved loin color and water holding capacity (Real et al., 2002). In the beef industry, product discoloration results in economic losses of $3.73 billion annually (Ramanathan et al., 2022). Due to the vastness of the pork industry, even small detriments to quality can have large economic impacts (Ellis et al., 2003); therefore, the current study investigated a

vitamin B_3 analog as a potential dietary countermeasure to antemortem stress to ameliorate some of those quality defects.

Carcass yields, including live weight, hot carcass weight, and wholesale cuts were not affected by NR supplementation. This was to be expected, as the supplementation was not intended to affect growth parameters and shows that NR supplementation is not detrimental to yields. Niacin, another vitamin B_3 compound, also had no effect on carcass weights in hogs (Real et al., 2002; Khan et al., 2013b), and neither did tryptophan, a precursor to niacin, in a study by Panella-Riera et al. (2008). Fat thickness and loin eye area were unaffected by NR supplementation which can also be considered to be a positive result, as body composition is not detrimentally affected. Tryptophan also had no effect on fat thickness (Panella-Riera et al., 2008). Real et al. (2002) also reported no differences in any fat thickness or loin eye area, but did note decreased carcass shrink, which was not observed in the present study. Supplementation of L-carnitine, another vitamin-like substance that improves mitochondrial function similar to NR (Gonzalez and Jackson, 2020), also did not affect pork affect live weight, dressing percentage, LEA, backfat, and cut out measurements (Han and Thacker, 2005; Pietruszka, 2009). To the authors' knowledge, no literature has investigated the effects of NR on pork, with the exception of a pilot study conducted by their own lab (Gonzalez et al., 2019); however, NR supplementation in other species also resulted in no change in overall body weight in an *in ovo* model (Gonzalez and Jackson, 2020) and postnatal models (Canto et al., 2012; Ryu et al., 2016).

Rapidly declining postmortem pH is largely accepted to be the cause of the quality defects associated with PSE (van Laack and Solomon, 1994). The PSE condition

can either be caused by rapid decline in pH in early hours postmortem causing increased protein denaturation (Briskey and Wismer-Pederson, 1961), or by a reduced ultimate pH reached by 24 hours caused by elevated muscle glycogen mainly caused by genetic mutations (Monin and Sellier, 1985; Lundstrom et al., 1996). Data from the present study cannot support that NR supplementation prevents rapid pH decline following a stress event, as no treatment affected pH for any of the four muscles measured. Real et al. (2002) also reported no differences in pH from niacin-supplemented carcasses at 45 minutes postmortem, which is in agreeance with the lack of differences at 1 h postmortem in the current study; however, Real et al. (2002) did find increased ultimate pH with increasing levels of niacin supplementation, which is conflicting with ultimate pH reported in the current study, but Real et al. (2002) did not subject pigs to a stress-event other than an typical approach to the slaughterhouse. The increased antemortem stress in the current study may be the reason no treatment effect is seen. All muscles in the current study had initial 1 h pH values that were less than those reported in Real et al. (2002), and ultimate pH values closer to the pH of the control in Real et al. (2002), but not comparable to the ultimate pH of carcasses from niacin-supplemented pigs. While muscles in the current study were not compared to each other, it seems that each muscle had different average starting pH at 1 h, indicating that we cannot compare pH between different muscles, but the 1 h pH of the *longissimus* in the current study was an average of 0.2 pH units less than the 45 min pH values reported in Real et al. (2002). Both Van der Wal et al. (1999) and Rosenvold et al. (2003) reported a 45 min postmortem *longissimus* pH value for non-stressed pigs 0.4 units greater than the average 1 h pH of the current study, but only 0.2 units greater for their stress model pigs. Interestingly, there

was a TRT × HR interaction for the *tensor fascia latae*, which is a muscle associated with locomotion. This interaction showed that at 1-h, *tensor fascia lataes* from the 45NR treatment had 4.6% smaller pH values than those in other carcasses, but at 2-h DRE *tensor fascia lataes* had 4.5% smaller pH values those from the CON, 30NR, and 45NR treatments, but those from 15NR were not different from any treatment. From 3-h on, no differences between treatment were observed. It is unclear why the two greater dose treatments resulted in a decreased pH in this muscle in the first two hours postmortem, but this could be because those treatments had greater muscle energy levels, and the fatigue-inducing event spent a greater proportion of the smaller dose pigs' energy, leaving the greater dose pigs with more mobilized glycogen. Approximately a 0.4 to 0.8 difference in pH in the first few hours postmortem constitutes a quality difference (Scheffler and Gerrard, 2007; S. Lonergan, 2012) and a difference of this magnitude is not seen in the current study.

Water-holding capacity can also be diminished by an increased rate of pH decline due to protein denaturation during rapid postmortem glycolysis (Offer, 1991). Therefore, the present study measured water-holding capacity using multiple analyses. Supplementation of NR did not impact water holding capacity measures, which is in contrast with niacin supplementation which was able to decrease drip loss by up to 1.2% without the addition of a stress event prior to slaughter (Real et al., 2002). Tryptophan, like NR, did not decrease drip loss; however tryptophan was not studied after a stress-inducing event (Paniella-Riera et al., 2008).

Treatment with NR did not affect objective or subjective lean color nor marbling on fabrication day. This is different from results reported after niacin supplementation,

which resulted in decreased loin eye L* and b* values, and increased subjective redness scores (Real et al, 2002), indicating darker loin eyes. Supplementation of L-carnitine also resulted in darker, redder loin eyes (Owen et al., 2001b; Han and Thacker, 2005). In accordance with the current results, though, tryptophan supplementation did not improve loin eye color or abundance of intramuscular fat (Panella-Riera et al., 2008).

Lighter lean color is a primary manifestation associated with PSE pork (Warris et al., 2000). The literature, for many years, documented meat color as one of the foremost factors consumers consider when purchasing meat in a retail setting (Cassens et al., 1989; Vierck et al., 2018). Additionally, premature discoloration can cost the meat industry money each year; therefore, pork color and stability were a primary focus of the present study. The day of display effects for all objective and subjective measure were expected, and resemble discoloration patterns documented in other studies (Sheard et al., 2000; Gonzalez et al., 2020). Nicotinamide riboside supplementation did not have meat color or color stability effects following the fatigue-inducing event used in this study. In a pilot study using NR supplementation (Gonzalez et al., 2019) demonstrated loin chops from barrows orally drenched with NR for a 13-d period had increased a* values and less discoloration than control barrows. The current study did not observe the same effects. These results were contrary to findings of Naruse et al. (1998) that demonstrated the ability of niacin to maintain color stability in lamb. While niacin and L-carnitine improved meat color initially, few data actually quantified their effects on color over time. There was likely no treatment effect for color stability in the current study due to outlier pigs in the 30NR treatment and the difficulty of attaining the same level of fatigue within each barrow during the performance test.

Metmyoglobin reducing activity is typically associated with color stability as meat has the inherent ability to reduce metmyoglobin to deoxymyoglobin. Deoxymyoglobin converts to oxymyoglobin, ultimately causing meat products to appear bright red which is associated with freshness (Reddy and Carpenter, 1991). Muscle NADH content increases postmortem muscle MRA through NADH-dependent reductases (Bekhit et al., 2003; Mancini et al., 2004). Because NAD+ is converted to NADH in the muscle, a supplement that increases NAD+ should be able to increase MRA in postmortem muscle, which has been demonstrated in beef through the lactate-NAD-lactate dehydrogenase system (Kim et al., 2006). While no data currently exists on NR supplementation, multiple studies proved NR increases NAD+ in the muscle of rats (Canto et al., 2012), humans (Martens et al., 2018), and poultry (Xu et al., 2021). The current study did not support the ability of NR supplementation to increase MRA through increased NADH as no treatment effect was observed on MRA. As mentioned previously, niacin proved to increase color stability (Naruse et al., 1998), which suggests increased MRA, but no assay was used in that particular study to measure MRA. Metmyoglobin reducing activity did decline over time in the current study, as expected and is consistent with results from other studies (Gonzalez et al., 2009; Paulk et al., 2014). The largest variance between treatment in the current study was approximately 5%, while Gonzalez et al. (2009) reported significant differences at 14%, and Paulk et al. (2014) reported significant differences at 10%. The smaller percent differences in the current study may be the reason no treatment effect is seen.

Oxygen consumption can also be correlated to meat color and color stability, as more active postmortem mitochondria are able to consume more oxygen, slowing down

the detrimental effect of oxygenation on meat throughout display (Madhavi and Carpenter, 1993). Nicotinamide riboside supplementation has proven to increase mitochondrial functionality in skeletal muscle in mice through NAD+ production (Canto et al., 2012; Khan et al., 2014), leading current researchers to investigate if NR has the same effect on postmortem pork muscle. Zhai et al. (2022) concluded NR-supplemented pork muscle had a slower decline in mitochondrial functionality from 0 to 24 h postmortem. In the current study, NR did not affect OC during the display period, which corroborates the pilot study that also demonstrated no effect on OC (Gonzalez et al., 2019). The lack of effect in both studies may be due to the supplementation period not being long enough to improve mitochondrial function to a degree that results in a significant difference between treatments.

No treatment was observed for WBSF values in the present study, in agreeance with Panella et al. (2005) who noted no difference in shear force values for chops from tryptophan-supplemented pigs. Castilha et al. (2016) observed a quadratic effect for shear force when pigs were supplemented with standardized ileal digestible tryptophan from 70 – 100 kg BW, where the second largest dose had the greatest shear force values, but the maximum dose exhibited the smallest shear force values, but all values reported were an average of 0.57 kg/f greater than those reported in the current study. The differences between studies could be due to the lack of postmortem aging in Castilha et al. (2016). In accordance with the current study, Waylan et al. (2003) observed no differences in thaw loss, cook loss, or shear force in the loin of pigs supplemented with L-carnitine from 45 to 107 kg BW, but values reported were between 0.27 to 0.93 kg/f greater than those in

the current study after a 6-d postmortem aging period, as compared to the 10-d aging in the current study. Further investigation is needed to determine in NR affects WBSF.

Proximate composition of pork chops was not affected by treatment in the present study, which corresponds to results from L-carnitine supplementation in hogs that resulted in no differences in protein or lipid deposition (Owen et al., 2001a). Somewhat in contrast, niacin supplementation increased loin eye marbling after 117-d supplementation period (Real et al., 2002); while this is not a measure of proximate composition, it could be indicative of greater fat content. From the proximate composition in the current study, NR supplementation does not increase chop moisture or intramuscular fat abundance, but this result is not surprising due to the much shorter supplementation period in the present study than reported in Real et al. (2002).

In a postnatal mouse model, NR supplementation increased CSA of type I muscle fibers after 28-d of supplementation (Seldeen et al., 2021) but not fiber CSA in an *in ovo* poultry model (Xu et al., 2021) as muscle fiber development is drastically different during prenatal and postnatal stages. The current study observed no effects on muscle fiber CSA. Comparably, sows supplemented with L-carnitine demonstrated no difference in muscle fiber CSA (Ramanau et al., 2006), but a longer NR supplementation period may have a different effect.

Due to data supporting NR's positive effect on mitochondria (Khan et al., 2014; Zhai et al., 2022), the authors of the current study hypothesized an increase in oxidative fiber types may reduce risk of PSE and increase color stability. This could not be supported, however, as no treatment effect was seen on fiber types. Contrarily, niacin supplementation to both hogs and sheep did prompt the shift of type II fibers to type I

(Khan et al., 2013a; Khan et al., 2013b); however, these studies supplemented animals for 3 and 4 weeks, respectively, while the present study only supplemented hogs for 10 d. The short supplementation period is likely the reason no fiber type shift was observed. The current study did observe comparable fiber type distributions in the *longissimus* muscle to those in other pork studies (Paulk et al., 2014; Noel et al., 2016). In agreeance with results observed in poultry reported by Xu et al. (2021), analysis of SDH staining in the current study revealed no differences in mitochondrial densities between fiber types in *longissimus* from NR-supplemented pigs and CON pigs. In agreeance, Alcocer et al. (2022) reported no difference between NR treatments in SDH intensity of the *biceps femoris* and *semitendinosus* muscle; however a treatment effect was seen for type IIA fibers in the *tensor fascia latae*, where *tensor fascia lataes* from the 45NR treatment stained more intensely than 30NR, indicating more dense mitochondria, and again pointing to an issue with the 30NR barrows. Potentially, this effect was seen in the *tensor fascia latae* and not in the *longissimus* because the *tensor fascia latae* muscle is more glycolytic (Sazili et al., 2005) and therefore the increase in oxidative capacity is more evident in this muscle.

The lack of positive effects observed in this study could be due to a number of factors. One important factor could be that hogs in the 45NR treatment, the greatest level of dietary inclusion, had decreased ADI in the period from d-5 to d-10 of supplementation (Alcocer et al., 2022). Developments in strategies to reduce any off flavors produced by NR, thereby encouraging pigs to ingest more of the compound, may permit the positive effects of NR supplementation that were expected in this study. Additionally, a fatigue-inducing event more representative of a trailer and lairage

experience that market hogs would endure may unveil more positive effect, as the authors believe the performance test used in the current study was more stressful than what pigs would endure in an industry setting prior to slaughter.

Positive Implications

While no significant differences were seen between chops, these results do not preclude the possibility of a positive effect if supplemented prior to other fatigue- or stress-inducing events. Certain treatment averages showed potentially promising results. Primarily, DRE chops had an average of 2.5% greater color scores than the CON chops during 8 out of 9 days in the retail display period. The 2.5% difference would only correspond to a less than a half point on the NPPC color scale, which is a small increase in redness, but appears to be enough to be noticed by the panelists. Changes in discoloration means were not as clear as subjective color score, but DRE chops were between 2 and 5% less discolored than CON chops on 6 of 9 days in the display period. Average loin chop color score numerical differences between the two greater NR doses (DRE and 45NR) and negative control (CON) chops could indicate a potential positive effect from NR supplementation in future studies.

Discoloration means for 45NR were between 3 and 4% less than CON on 4 of 9 days. Interestingly, discoloration means were greatest on most days for chops from pigs treated with the moderate level of NR dietary inclusion, 30NR. This same trend, of composition traits improving with NR supplementation, with the exception of 30NR, occurred in multiple measurements throughout the present study. It is possible that this is due to confounding factors such as outlier pigs that did not cooperate throughout the performance test being in the 30NR treatment group, exacerbating the stress effects even

more. It is also possible that these outliers prevented a treatment main effect from being observed.

L* values were numerically different between the DRE and CON treatment groups such that average L* values of the DRE treatment group were 2.7% lower than the average L* values of the CON treatment group over the entire display period. This numerical difference indicates that DRE pork chops, on average, were slightly darker than CON chops. Additionally, a* value was, on average, 3% numerically greater in DRE chops on 8 of 9 days in the display period, with differences of up to 5% on the final day of display, suggesting those chops were slightly redder. This is supported by the pilot study, as a significant increase in a* value of 5% on average for NR chops over an 8-d display period was observed (Gonzalez et al., 2019). Likely, the reason the average percent increase in the pilot study was greater than the current study is that barrows in the pilot study were allowed a 3-d rest period prior to harvest. This is a probable reason no treatment effect was observed in the present study. In objective color measurements, the same trend was seen with chops from the 30NR treatment: chops in this treatment has second greatest L* values, second only to those in the CON treatment, and had the smallest a* values on every day of the display period. This indicates the 30NR chops were the lightest and least red.

As previously mentioned, chops from DRE group had an average of approximately 4% greater MRA than CON chops on the three days it was measured during the display period. Other studies (Gonzalez et al., 2009; Paulk et al., 2014) reported significant differences in MRA at 14% and 10%, respectively. Based on these studies, it is likely that increases in the present study did not result in treatment effects

because the increases were not large enough. The numerical increase in MRA could be due to an increase in NADH in postmortem muscle.

Chops did show numerical differences in OC on d-0 and d-8, but not d-4. Chops from DRE had approximately 11% greater OC on d-0 and 7% greater OC on d-8 than CON chops. Considering these numerical differences, and the proven positive effect NR supplementation has had on mitochondria (Canto et al., 2012; Khan et al., 2014), this could suggest the potential for NR to increase postmortem mitochondrial functionality and, in turn, oxygen consumption. Increased postmortem mitochondrial activity would slow meat discoloration (Madhavi and Carpenter, 1993). Rosenvold and Anderson (2003) concluded that although antemortem stress may not have affected 24 h color, the stressors may have caused denaturation of proteins that compete with myoglobin for oxygen, which could have caused increased bloom, but decreased color stability over storage, indicating the CON chops may have had decreased OC and subsequently decreased color stability and decreased L* value compared to DRE chops.

No treatment effect was observed for WBSF values in the present study, in agreeance with Panella et al. (2005), who noted no difference in shear force values for chops from tryptophan-supplemented pigs. Additionally, while WBSF values were not affected by treatment, all chops from NR-treated pigs had smaller peak force values than those from the CON treatment. Chops from the oral drench treatment had the smallest average peak force values. While not significant, this trend was interesting, as many supplementation studies did not affect shear force values (Waylan et al., 2003; Panella et al., 2005).

It is possible that no treatment effects were observed in the current study due to confounding factors, including uncooperative pigs or decreased intake. The effect of the NR in the current study may also have been dampened by the fatigue inducing event, as it was different from the pilot study and had no rest period. Considering the aforementioned positive trends and the potential for confounding factors, continued research into NR supplementation in market hogs may uncover beneficial effects.

Table 1. Effect of nicotinamide riboside supplementation on pork carcass yields[1]

	Treatment						
	CON	15NR	30NR	45NR	DRE	SEM	*P*-value
Weights, kg							
Live weight	131.5	131.0	130.5	131.0	131.0	3.0	0.99
Hot carcass weight	99.5	100.0	99.0	100.0	99.0	2.2	0.99
Cold side weight	49.0	49.5	49.5	50.0	49.0	0.8	0.99
Yields, %							
Dressing	75.7	76.1	76.1	75.6	75.6	0.7	0.84
Shrink	3.0	2.2	2.0	1.2	2.1	0.7	0.36

[1]One hundred barrows were supplemented one of five different levels of nicotinamide riboside orally: 0 mg•hd^{-1}•kg body weight^{-1} (CON), 15 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (15NR), 30 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (30NR), 45 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (45NR), and 45 mg•hd^{-1}•kg body weight^{-1} provided in an oral drench form (DRE) for 11 days prior to a fatigue-inducing performance test and then harvested.

Table 2. Effect of nicotinamide riboside supplementation on pork carcass quality and yield parameters[1]

	Treatment						
	CON	15NR	30NR	45NR	DRE	SEM	*P*-value
Fat thickness, cm							
First rib	4.5	4.7	4.2	4.3	4.4	0.2	0.21
10th rib	2.6	2.5	2.4	2.6	2.4	0.1	0.82
Last rib	3.0	3.3	3.1	2.9	3.1	0.2	0.42
Loin eye area, cm^2	18.5	18.0	19.7	18.8	18.1	0.7	0.36
Color[2]							
NPPC color	2.65	2.80	2.65	2.92	2.90	0.16	0.36
NPPC marbling score	1.73	2.01	2.17	1.98	2.07	0.15	0.23
Japanese lean color score	2.6	2.39	2.28	2.45	2.47	0.16	0.71
Japanese marbling color	1.21	1.12	1.26	1.14	1.28	0.07	0.20
Japanese subcutaneous fat color	1.38	1.50	1.60	1.36	1.41	0.12	0.27
L*	64.64	63.23	64.60	62.59	62.94	0.94	0.15
a*	19.07	19.14	18.79	19.46	19.26	0.40	0.65
b*	18.39	18.25	18.11	18.24	18.15	0.35	0.93

[1]One hundred barrows were supplemented one of five levels of nicotinamide riboside orally: 0 mg•hd^{-1}•kg body weight^{-1} (**CON**), 15 mg•hd^{-1}•kg body weight^{-1} in feed (**15NR**), 30 mg•hd^{-1}•kg body weight^{-1} in feed (**30NR**), 45 mg•hd^{-1}•kg body weight^{-1} in with (**45NR**), and 45 mg•hd^{-1}•kg body weight^{-1} in an oral drench form (**DRE**) for 11 days prior to a fatigue-inducing performance test and then harvested. After chilling 24 h, carcasses were ribbed, allowed to bloom for 30 min, and carcass quality measurements collected.

[2]NPPC = National Pork Producers' Council. Based on 6 point scale (NPPC, 1999). Japanese color tiles were used for Japanese score (Nakai et al., 1975) and scores were averaged from three researchers.

Table 3. Effect of nicotinamide riboside supplementation on pork side cutting yields[1]

	Treatment[2]						
	CON	15NR	30NR	45NR	DRE	SEM	*P*-value
Wholesale weights, kg							
Bone-in Boston Butt (IMPS #406)	2.31	2.26	2.33	2.32	2.25	0.42	0.91
Bone-in picnic shoulder (IMPS #405)	4.14	4.04	4.18	4.06	4.13	0.15	0.88
Trimmed boneless loin (IMPS #413)	5.03	4.99	5.16	5.16	5.04	0.37	0.86
Baby back ribs	0.64	0.66	0.65	0.64	0.66	0.04	0.88
Spare ribs (IMPS #416)	1.67	1.63	1.66	1.60	1.64	0.07	0.91
Whole belly, skinless (IMPS #409)	7.25	7.29	7.39	7.15	7.19	0.22	0.94
Ham, skin-off (IMPS #402)	10.10	9.92	9.96	10.20	9.86	0.23	0.73
Tenderloin, trimmed (IMPS #415)	1.03	0.75	0.70	0.81	0.73	0.13	0.34
Feet (IMPS #420, 420A)	5.83	5.87	5.88	5.60	5.90	0.66	0.77
Fat (IMPS #406C, 410C)	3.61	3.59	3.43	3.52	3.49	0.18	0.87
Skin	3.24	3.29	2.91	2.98	3.12	0.24	0.60
Trim (IMPS #418)	0.91	0.99	1.06	1.04	0.92	0.11	0.75
Bones	3.49	3.56	3.60	3.72	3.51	0.18	0.86
Offal	0.43	0.34	0.43	0.36	0.43	0.08	0.82
Cut loss, %	2.68	3.71	3.12	3.51	2.88	1.15	0.81

[1]One hundred barrows were supplemented one of five different levels of nicotinamide riboside orally: 0 mg•hd^{-1}•kg body weight^{-1} (CON), 15 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (15NR), 30 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (30NR), 45 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (45NR), and 45 mg•hd^{-1}•kg body weight^{-1} provided in an oral drench form (DRE) for 11 days prior to a fatigue-inducing performance test and then harvested. After chilling for 24 h, carcass left sides were fabricated into wholesale cuts. Weights are reported in kg.

Table 4. Effect of nicotinamide riboside supplementation on pork water holding measures[1]

	Treatment						
	CON	15NR	30NR	45NR	DRE	SEM	*P*-value
Swelling, %	65.83	73.83	72.40	75.09	84.77	12.34	0.55
Expressible moisture, %	14.92	14.62	15.72	15.88	15.84	1.65	0.74
Drip loss, %	2.23	1.83	2.04	2.17	2.31	1.01	0.37

[1]One hundred barrows were supplemented one of five different levels of nicotinamide riboside orally: 0 mg•hd^{-1}•kg body weight^{-1} (CON), 15 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (15NR), 30 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (30NR), 45 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (45NR), and 45 mg•hd^{-1}•kg body weight^{-1} provided in an oral drench form (DRE) for 11 days prior to a fatigue-inducing performance test and then harvested. After chilling for 24 h, carcass left sides were fabricated, and whole boneless loins were aged in vacuum bags for 10 d in the dark. Loins were then halved immediately posterior to the *spinalis dorsi*. Anterior to where the loin was halved, four 2.54-cm chops were cut and labeled 9 (most anterior) through 12 (most posterior), and trimmed of external fat. Chop 9 was used for drip loss analysis, chip 10 for expressible moisture analysis of water holding, and chop 11 was designated for water binding analysis on that day.

Table 5. Effect of nicotinamide riboside supplementation on cooking parameters and Warner-Bratzler shear force[1]

	Treatment						
	CON	15NR	30NR	45NR	DRE	SEM	*P*-value
Thaw loss, %	3.84	5.90	3.71	3.83	4.00	1.34	0.46
Cook loss, %	22.82	21.43	21.49	21.52	22.33	1.02	0.78
Cook time, s	198.70	181.15	195.15	210.72	199.31	9.46	0.25
Average peak force, kg/f	2.50	2.29	2.39	2.36	2.22	0.16	0.62

[1]One hundred barrows were supplemented one of five different levels of nicotinamide riboside orally: 0 mg•hd^{-1}•kg body weight^{-1} (CON), 15 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (15NR), 30 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (30NR), 45 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (45NR), and 45 mg•hd^{-1}•kg body weight^{-1} provided in an oral drench form (DRE) for 11 days prior to a fatigue-inducing performance test and then harvested. Loins were aged 10 days in vacuum bags, and halved posterior to the *spinalis dorsi*. Chop 6 from the posterior half was frozen at -20°C until thawed for Warner-Bratzler shear force analysis.

Table 6. Effect of nicotinamide riboside supplementation on pork loin chop proximate composition[1]

	Treatment						
	CON	15NR	30NR	45NR	DRE	SEM	*P*-value
Moisture, %	70.58	70.01	69.08	69.20	73.69	1.68	0.26
Fat, %	4.63	4.75	5.58	5.07	5.19	0.43	0.40
Protein, %	23.76	23.82	23.76	24.72	23.40	0.37	0.15

[1]One hundred barrows were supplemented one of five different levels of nicotinamide riboside orally: 0 mg•hd^{-1}•kg body weight^{-1} (CON), 15 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (15NR), 30 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (30NR), 45 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (45NR), and 45 mg•hd^{-1}•kg body weight^{-1} provided in an oral drench form (DRE) for 11 days prior to a fatigue-inducing performance test and then harvested. Loins were aged 10 days in vacuum bags, and halved posterior to the *spinalis dorsi*. The most anterior of four chops cut from the anterior half was frozen at -20°C until used for proximate analysis.

Table 7. Effect of nicotinamide riboside supplementation on pork *longissimus dorsi* muscle fiber type characteristics[1]

	Treatment						
	CON	15NR	30NR	45NR	DRE	SEM	*P*-value
Fiber Type, %							
Type I	8.6	9.7	8.7	9.4	8.2	1.1	0.89
Type IIA	28.9	25.7	27.3	27.3	28.5	2.2	0.76
Type IIX	12.2	12.3	13.5	12.1	11.1	1.1	0.58
Type IIB	50.1	51.0	51.9	52.3	52.1	2.3	0.96
Cross-sectional area, μm^2							
Type I	3405	2904	3095	3226	3474	219	0.26
Type IIA	3579	3176	3179	3572	3830	269	0.32
Type IIX	4291	4268	4127	4681	4844	285	0.22
Type IIB	4571	4032	3979	4136	4264	279	0.22
SDH intensity, a.u.[2]							
Type I	114.5	118.9	124.9	126.4	115.5	12.8	0.82
Type IIA	139.2	138.7	153.2	147.0	132.2	11.0	0.32
Type IIX	152.2	150.7	164.0	162.5	145.9	11.5	0.39
Type IIB	166.5	166.4	177.3	174.0	160.9	11.4	0.47

[1]One hundred barrows were supplemented one of five levels of nicotinamide riboside orally: 0 $mg \cdot hd^{-1} \cdot kg$ body $weight^{-1}$ (**CON**), 15 $mg \cdot hd^{-1} \cdot kg$ body $weight^{-1}$ in feed (**15NR**), 30 $mg \cdot hd^{-1} \cdot kg$ body $weight^{-1}$ in feed (**30NR**), 45 $mg \cdot hd^{-1} \cdot kg$ body $weight^{-1}$ in with (**45NR**), and 45 $mg \cdot hd^{-1} \cdot kg$ body $weight^{-1}$ in an oral drench form (**DRE**) for 11 days prior to a fatigue-inducing performance test and then harvested. After chilling 24 h, carcasses were ribbed, allowed to bloom for 30 min, and carcass quality measurements collected. The medial portion of the first chop cut posterior to the *spinalis dorsi* was used for immunohistochemical analyses.

[2]SDH = succinate dehydrogenase: 0 = most intense stain and 250 = least intense

Figure 1. Effect of nicotinamide riboside supplementation on postmortem pH of pork a) *semitendinosus*, b) *biceps femoris*, c) *tensor fascialatae*, and d) *longissimus lumborum*. One hundred barrows were supplemented one of five different levels of nicotinamide riboside orally: 0 mg•hd^{-1}•kg body weight^{-1} (CON), 15 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (15NR), 30 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (30NR), 45 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (45NR), and 45 mg•hd^{-1}•kg body weight^{-1} provided in an oral drench form (DRE) for 11 days prior to a fatigue-inducing performance test and then harvested. Carcass pH was measured at 1, 2, 3, 4, 5, 6, 12, and 24 h postmortem. Means are compared within hour.

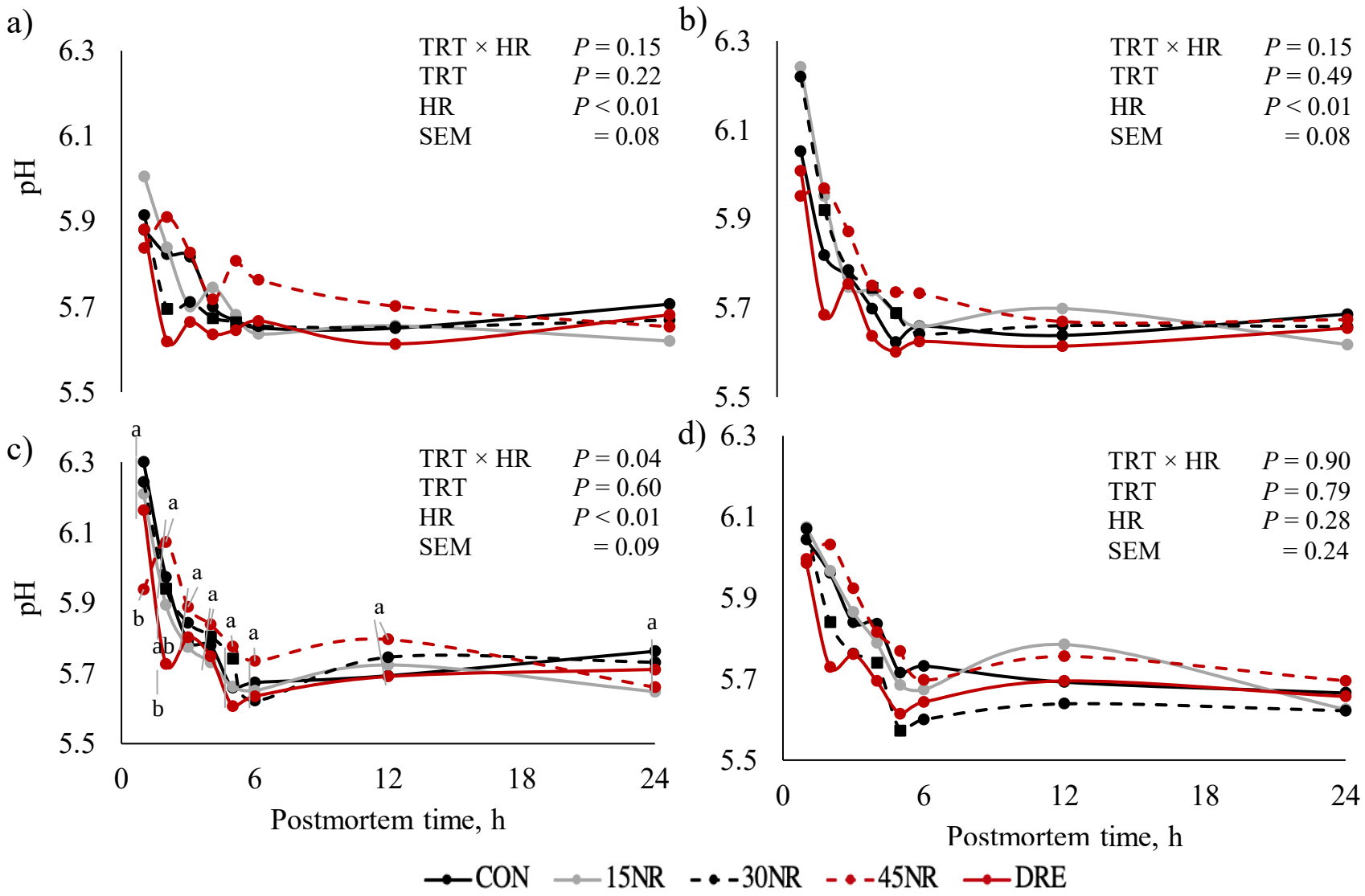

a)
b)
c)
d)
pH
6.3
6.1
5.9
5.7
5.5
TRT × HR P = 0.15
TRT P = 0.22
HR P < 0.01
SEM = 0.08
TRT × HR P = 0.15
TRT P = 0.49
HR P < 0.01
SEM = 0.08
TRT × HR P = 0.04
TRT P = 0.60
HR P < 0.01
SEM = 0.09
TRT × HR P = 0.90
TRT P = 0.79
HR P = 0.28
SEM = 0.24
0
6
12
18
24
Postmortem time, h
CON
15NR
30NR
45NR
DRE

Figure 2. Effect of nicotinamide riboside supplementation on subjective pork chop color during simulated retail display. One hundred barrows were supplemented one of five different levels of nicotinamide riboside orally: 0 mg•hd^{-1}•kg body weight^{-1} (CON), 15 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (15NR), 30 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (30NR), 45 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (45NR), and 45 mg•hd^{-1}•kg body weight^{-1} provided in an oral drench form (DRE) for 11 days prior to a fatigue-inducing performance test and then harvested. After 10 d of aging, whole boneless loins were halved immediately posterior to the *spinalis dorsi*. Posterior to where the loin was halved, eight 2.54-cm chops were cut. Chop 4 was used for simulated retail display objective and subjective color-stability. Lean color was evaluated on a 100-point line scale with anchor points at the minimum, maximum, and middle of the scale. A score of 1 was equivalent to a National Pork Producers Council (NPPC) color score of 1.0 (pale pinkish gray to white), a 50.5 = NPPC 3.5 (between reddish pink and dark reddish pink), and 100 = NPPC 6.0 (dark purplish red). Discoloration was measured on a 100-point line scale, with anchors at 0, 50, and 100. A score of 0 = 0% discolored, 50 = 50% discolored, and 100 = 100% discolored. Panelists' scores were averaged daily.

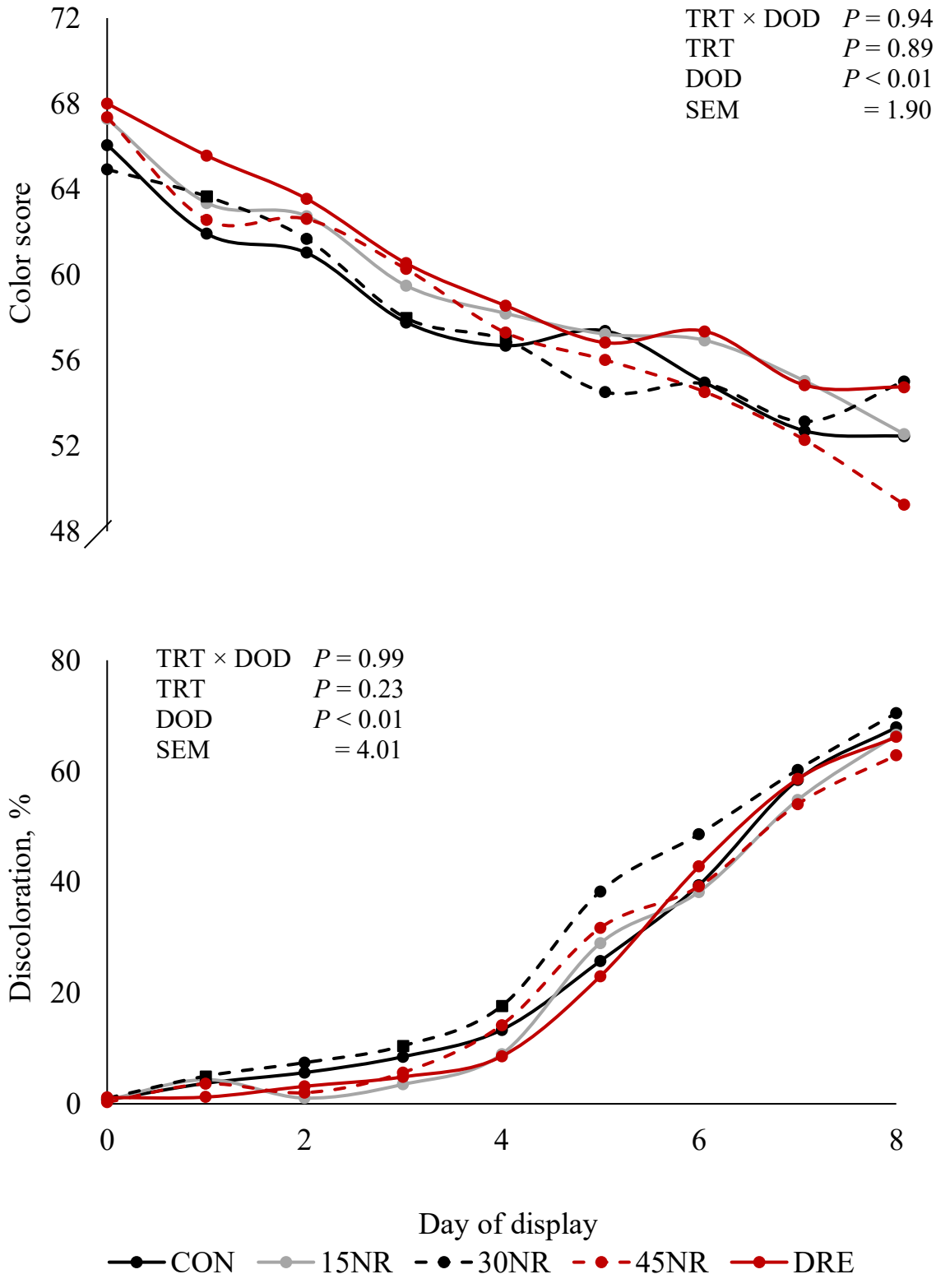

TRT × DOD P = 0.94
TRT P = 0.89
DOD P < 0.01
SEM = 1.90
Color score
72
68
64
60
56
52
48
TRT × DOD P = 0.99
TRT P = 0.23
DOD P < 0.01
SEM = 4.01
Discoloration, %
80
60
40
20
0
0
2
4
6
8
Day of display
CON
15NR
30NR
45NR
DRE

Figure 3. Effect of nicotinamide riboside supplementation on objective pork chop color during simulated retail display. One hundred barrows were supplemented one of five different levels of nicotinamide riboside orally: 0 mg•hd^{-1}•kg body weight^{-1} (CON), 15 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (15NR), 30 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (30NR), 45 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (45NR), and 45 mg•hd^{-1}•kg body weight^{-1} provided in an oral drench form (DRE) for 11 days prior to a fatigue-inducing performance test and then harvested. After 10 d of aging, whole boneless loins were halved immediately posterior to the *spinalis dorsi*. Posterior to where the loin was halved, eight 2.54-cm chops were cut. Chop 4 was used for simulated retail display objective and subjective color-stability. Chops were scanned with a handheld spectrophotometer daily for objective color values. The average of three scans was used for each chop.

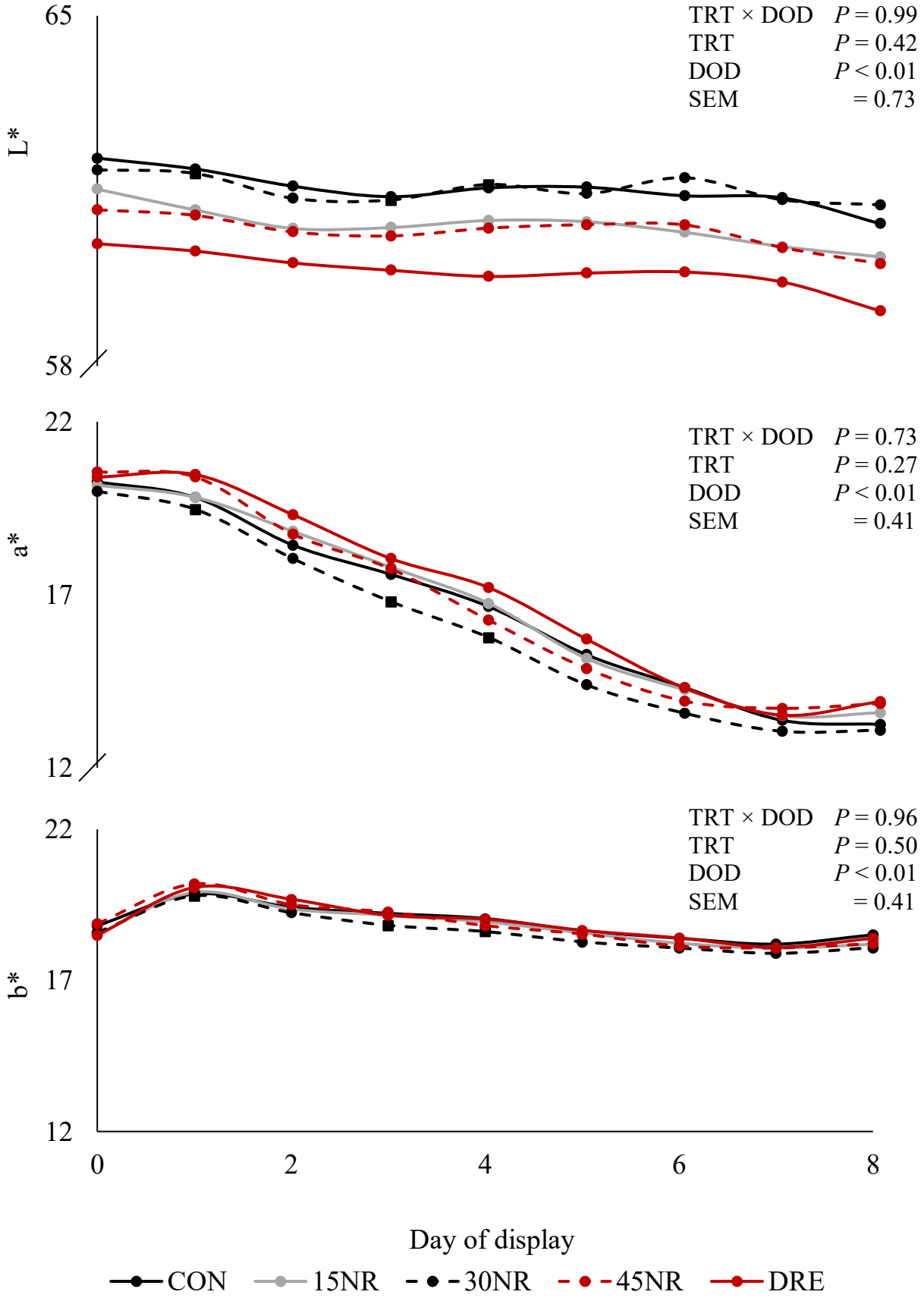

L*
65
58
TRT × DOD P = 0.99
TRT P = 0.42
DOD P < 0.01
SEM = 0.73
a*
22
17
12
TRT × DOD P = 0.73
TRT P = 0.27
DOD P < 0.01
SEM = 0.41
b*
22
17
12
TRT × DOD P = 0.96
TRT P = 0.50
DOD P < 0.01
SEM = 0.41
0
2
4
6
8
Day of display
CON
15NR
30NR
45NR
DRE

Figure 4. Effect of nicotinamide riboside supplementation on surface metmyoglobin, deoxymyoglobin, and oxymyoglobin during simulated retail display. One hundred barrows were supplemented one of five different levels of nicotinamide riboside orally: 0 mg•hd^{-1}•kg body weight^{-1} (CON), 15 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (15NR), 30 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (30NR), 45 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (45NR), and 45 mg•hd^{-1}•kg body weight^{-1} provided in an oral drench form (DRE) for 11 days prior to a fatigue-inducing performance test and then harvested. After 10 d of aging, whole boneless loins were halved immediately posterior to the *spinalis dorsi*. Posterior to where the loin was halved, eight 2.54-cm chops were cut. Chop 4 was used for simulated retail display objective and subjective color-stability. Chops were scanned daily with a handheld spectrophotometer, and spectral data was used to estimate metmyoglobin, deoxymyoglobin, and oxymyoglobin using Krzywicki (1979) equations.

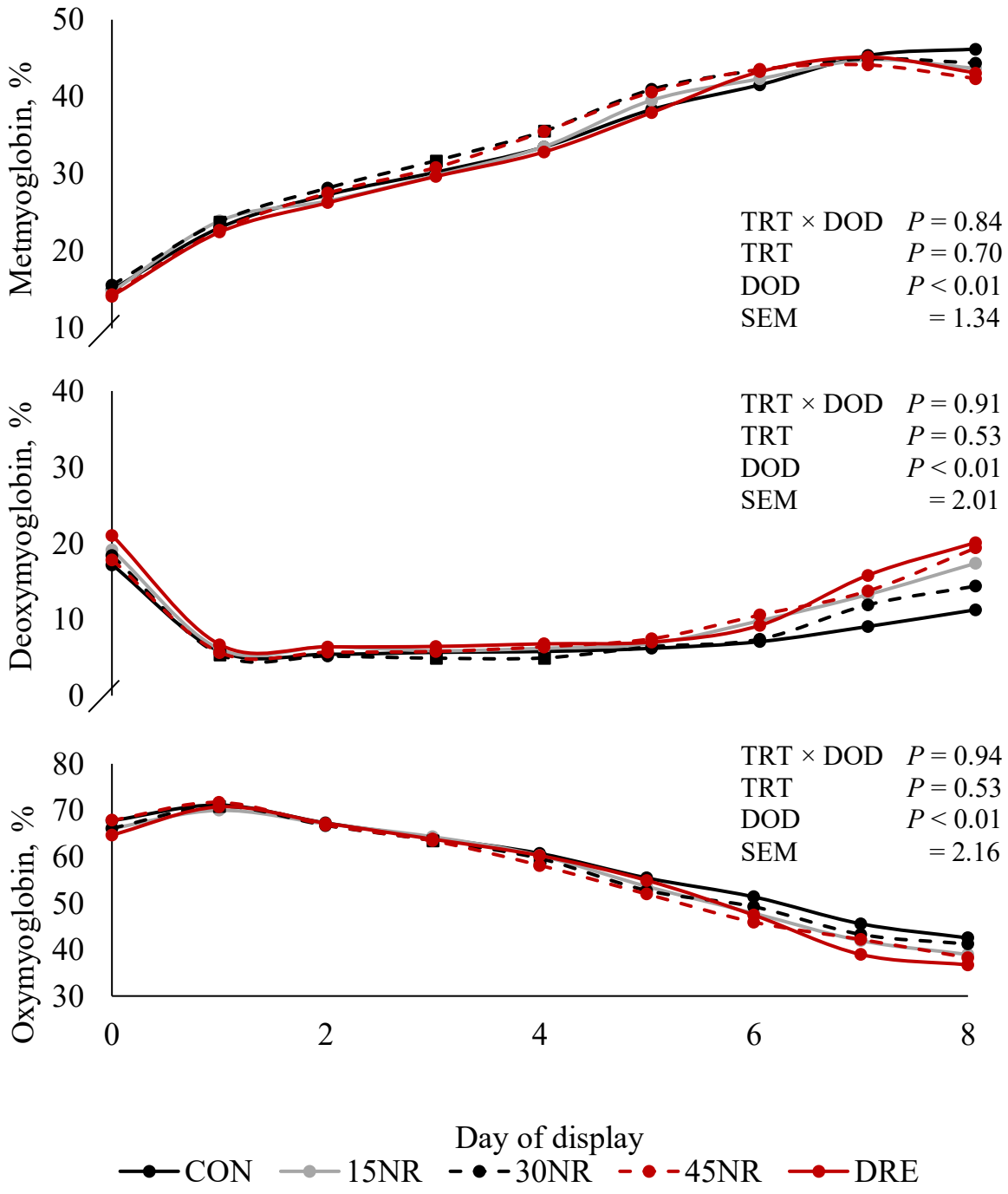

Metmyoglobin, %
50
40
30
20
10
TRT × DOD P = 0.84
TRT P = 0.70
DOD P < 0.01
SEM = 1.34
Deoxymyoglobin, %
40
30
20
10
0
TRT × DOD P = 0.91
TRT P = 0.53
DOD P < 0.01
SEM = 2.01
Oxymyoglobin, %
80
70
60
50
40
30
TRT × DOD P = 0.94
TRT P = 0.53
DOD P < 0.01
SEM = 2.16
0
2
4
6
8
Day of display
CON
15NR
30NR
45NR
DRE

Figure 5. Effect of nicotinamide riboside supplementation on chroma and hue angle during simulated retail display. One hundred barrows were supplemented one of five different levels of nicotinamide riboside orally: 0 mg•hd^{-1}•kg body weight^{-1} (CON), 15 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (15NR), 30 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (30NR), 45 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (45NR), and 45 mg•hd^{-1}•kg body weight^{-1} provided in an oral drench form (DRE) for 11 days prior to a fatigue-inducing performance test and then harvested. After 10 d of aging, whole boneless loins were halved immediately posterior to the *spinalis dorsi*. Posterior to where the loin was halved, eight 2.54-cm chops were cut. Chop 4 was used for simulated retail display objective and subjective color-stability. Chops were scanned daily for objective color values, and spectral data was used to determine chroma and hue angle.

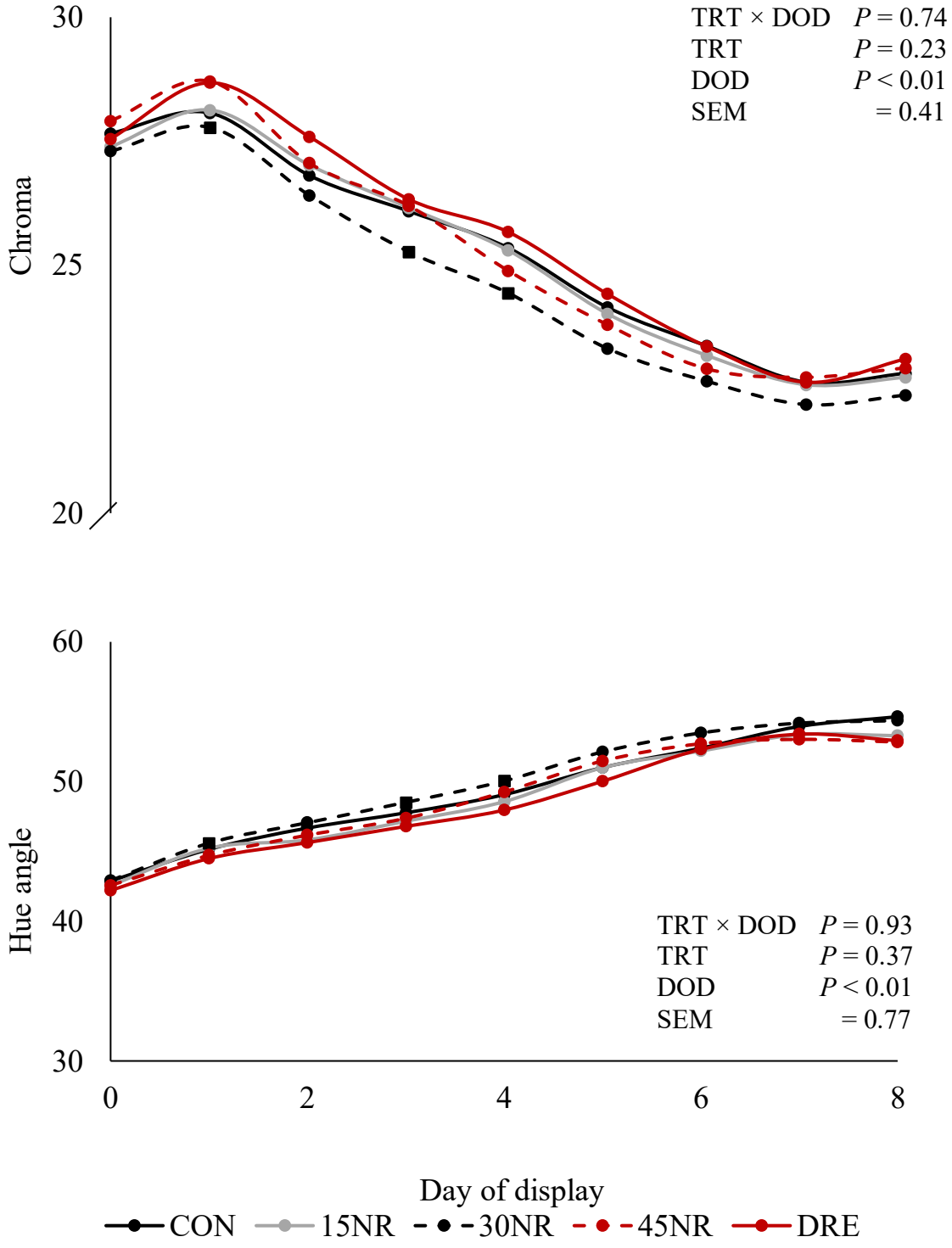
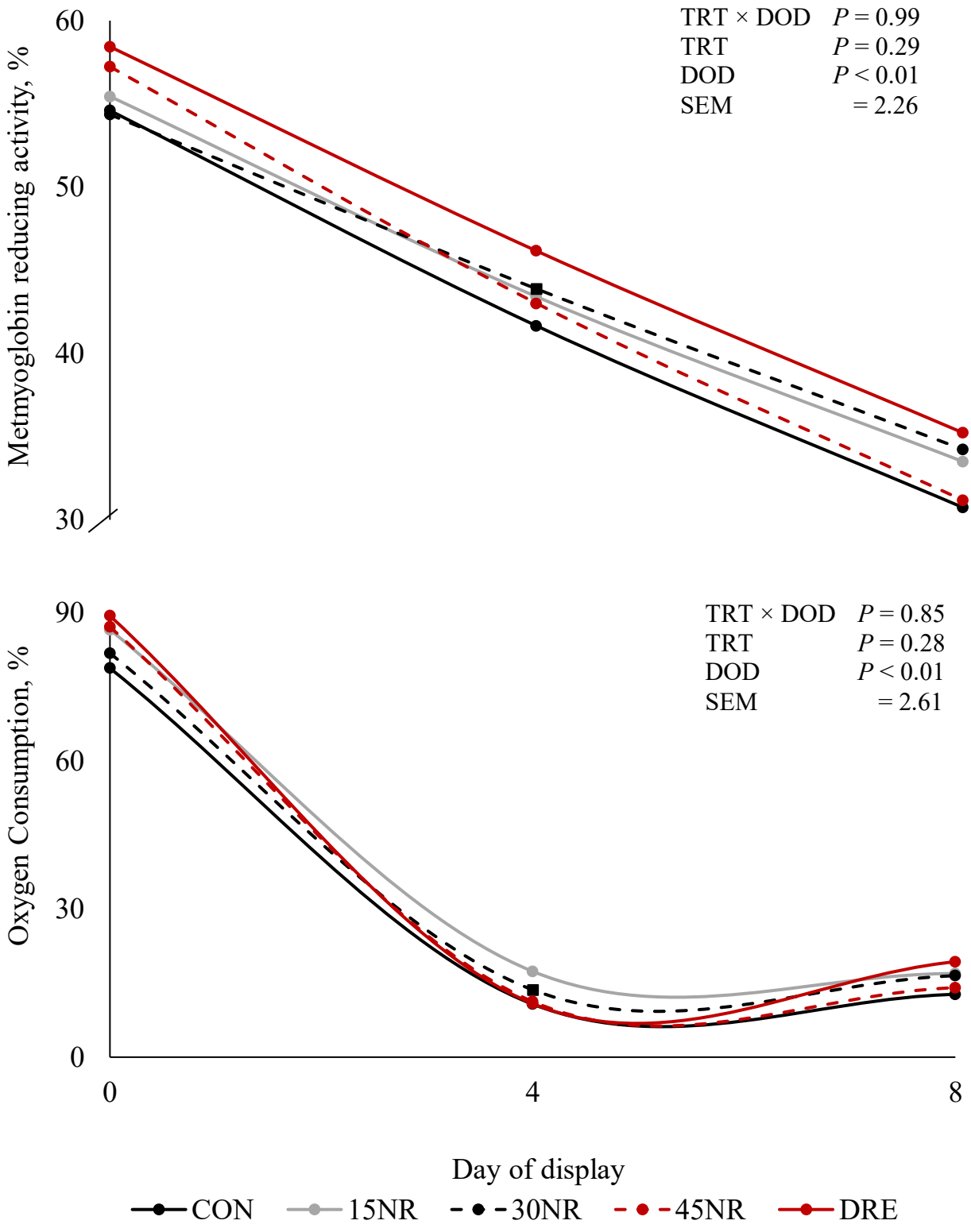

30
25
20
Chroma
TRT × DOD P = 0.74
TRT P = 0.23
DOD P < 0.01
SEM = 0.41
60
50
40
30
Hue angle
TRT × DOD P = 0.93
TRT P = 0.37
DOD P < 0.01
SEM = 0.77
0
2
4
6
8
Day of display
CON
15NR
30NR
45NR
DRE

Figure 6. Effect of nicotinamide riboside supplementation on metmyoglobin reducing activity (MRA) and oxygen consumption (OC). One hundred barrows were supplemented one of five different levels of nicotinamide riboside orally: 0 mg•hd^{-1}•kg body weight^{-1} (CON), 15 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (15NR), 30 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (30NR), 45 mg•hd^{-1}•kg body weight^{-1} mixed in with feed (45NR), and 45 mg•hd^{-1}•kg body weight^{-1} provided in an oral drench form (DRE) for 11 days prior to a fatigue-inducing performance test and then harvested. After 10 d of aging, whole boneless loins were halved immediately posterior to the *spinalis dorsi*. Posterior to where the loin was halved, eight 2.54-cm chops were cut. Chops 2 and 3 were halved and placed in coffin style retail display case for d-0 or -4 MRA and OC, and chop 4 and 5 were used for d-8 MRA and OC.


TRT × DOD P = 0.99
TRT P = 0.29
DOD P < 0.01
SEM = 2.26
Metmyoglobin reducing activity, %
60
50
40
30
TRT × DOD P = 0.85
TRT P = 0.28
DOD P < 0.01
SEM = 2.61
Oxygen Consumption, %
90
60
30
0
0
4
8
Day of display
CON
15NR
30NR
45NR
DRE

CHAPTER 4

CONCLUSIONS AND IMPLICATIONS

This study demonstrates no positive effects of NR-supplementation on fresh pork color, color stability, or other composition measures when pigs are greatly and acutely stressed before harvest. Supplementation of finishing market hogs with 15, 30, or 45 mg•hd^{-1}•kg body weight^{-1} for 10 d did not improve any attributes measured when compared with hogs that were not supplemented with NR. Fortunately, NR-supplementation was not detrimental to carcass yields, including dressing percentage, shrink percentage, back fat, loin eye area, or marbling. This indicates that supplementing finishing hogs with NR would not cause economic losses associated with decreased yields. Color stability and increased redness of chops were of primary interest in the current study, due to the ability of NR to improve muscle NAD+ content mitochondrial function; however, no NR treatments exhibited any differences in color on fabrication day, or color and discoloration during 8-d of simulated retail display. The NR supplementation also did not improve water holding capacity, Warner-Bratzler shear force, proximate composition, fiber type distribution, fiber type percentage, or SDH staining.

Interestingly, there were numerical improvements among some of the NR-treated carcasses in the current study. Confounding factors may have prevented NR from having an effect on color stability and other measures. Primarily, ADI was decreased from d-5 to d-10 of supplementation in one of the supplemented groups as reported in Alcocer et al. (2022), potentially due to the bitter flavor of the supplement. In multiple variables there was a

numerical trend in which one or more supplemented groups trended toward more desirable measurements compared to the control, including fabrication day color, display color, discoloration, L*, a*, MRA, OC, and WBSF. In many of these measurements, the middle level of supplementation (30NR) did not follow the trends, indicating there may have been confounding issues with hogs in that treatment. Considering these numerical trends, additional research using different supplementation methods and different fatigue-inducing events, including trailer transportation or simulated transportation, are necessary and may demonstrate positive impacts on pork color stability. Investigating feed samples with NR added, or analyzing tissue of fecal matter of the barrows to ensure the NR is active, consumed, and digested may be necessary in future studies. Currently, supplementation of NR to market hogs is not a viable method for the pork industry to use to ameliorate pork chop color stability or composition, but further investigation and developments in research methodology may reveal it to be one in the future.

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