EFFECTS OF VITAMIN B3 DOSE ON MARKET BARROW FATIGUE ONSET AND GAIT

by

HANNA MARIE ALCOCER

(Under the Direction of John Michael Gonzalez)

ABSTRACT

Fatigued pig syndrome is a swine industry problem. The objective of this study was to evaluate increasing dietary vitamin B3 dose effects on delaying muscle fatigue onset. Barrows were assigned one of five B3 doses in a swine finishing diet. After 10 d of supplementation, barrows were subjected to a performance test until fatigued. Intake decreased in pigs fed greatest B3 dose. Pigs supplemented greatest dose through drench solution run time and run duration increased during the performance test. Supplemented barrows had decreased creatine kinase and lactate blood concentrations compared to un-supplemented barrows pre- and post-fatigue. There were no treatment differences for gait mat analyses; however, there were changes between pre- and post- fatigue for velocity, stride time, cycle time, cadence, and cycle length. While not significant, data appears to indicate vitamin B3 may be a countermeasure to fatigue with potential for greater improvements through increased supplementation period.

INDEX WORDS: *fatigue, muscle fiber, nicotinamide riboside, barrow*

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## DEDICATION

I would like to dedicate this thesis to my fiancé, Josh. Without your invaluable support throughout my career I would not be where I am today. You push me to go outside of my comfort zone, thus making me a better student and individual. I cannot imagine where I would be in my life without you.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

# CHAPTER 1

# INTRODUCTION

Fatigued pig syndrome can be used to characterize non-deceased, non-ambulatory, and physically, non-injured pigs arriving at the abattoir demonstrating typical signs of fatigue including open-mouth breathing, muscle tremors, and increased reddening of the skin (Benjamin, 2005; Ritter et al., 2009). Typically, fatigued pigs recover during a 2 to 3 h lairage period after transportation to the abattoir; however, many pigs die before they recover (Hamilton et al., 2004; Ritter et al., 2005). Ritter et al. (2009) summarized 23 field studies between 2000 and 2007 and reported the percentage of transport losses attributed to fatigued pigs between 2009 and 2012. In 2009, 0.44% of pigs were classified as fatigued while that number increased to 0.66% by 2020 indicating this is a growing issue (Ritter et al., 2020). It is estimated dead and non-ambulatory pigs cost the U.S. swine industry $46 million (UDS) annually in 2009, with values increasing to $88 million in 2020 associated with increasing loss percentage (Ritter et al., 2020). Numerous environmental factors including season, trailer density, sorting methods, and feed withdrawal times were studied to understand fatigue in response to stress sources in pigs (Smith et al., 1976; Fitzgerald et al., 2009; Gesing et al., 2010; Doonan et al., 2014); however, biological stress indicators and mechanisms related to the muscle fatigue portion of pig fatigability have limited research available.

Internal substrates related to muscle fatigue include pyridine nucleotides, nicotinamide adenine dinucleotide (**NAD**+) and nicotinamide adenine dinucleotide + hydrogen (**NADH**). These nucleotides are coenzymes in muscle mitochondria which provide oxidoreductive power to the electron transport chain which is responsible for ATP production (Bogan and Brenner, 2008; Egan and Zierth, 2013; Li and Suave, 2015). Glycolysis production of NADH is utilized in

the electron transport chain for both NADH and ATP production. In past studies, increased NAD+ levels accompanied by decreased NADH levels were observed following exercise training in both rats and canines (Jobsis and Stainsby, 1968; Duboc et al., 1988). The levels of NAD+ in resting muscles are seen to be greatest in mitochondria dense fibers such as type I muscle fibers (Graham et al., 1978; Ren et al., 1988), which could indicate fiber type composition influences NAD+ levels. With the influence mitochondria concentration has on NAD+ and NADH levels, manipulation of mitochondrial biogenesis or efficiency could increase potential ATP production and fatigue resistance.

Supplemental nicotinamide riboside (**NR**), a naturally occurring vitamin B3 analogue, was studied in mice and humans and showed NR positively affected varying muscle groups' NAD+ concentrations (Canto et al., 2012; Zhang et al., 2016; Airhart et al., 2017; Conze et al., 2019). Khan et al. (2014) demonstrated NR supplemented mice, predisposed to mitochondrial myopathy, resulted in increased mitochondrial DNA, indicating increased energy production potential through mitochondrial biogenesis. These results are ideal as NAD+ has a direct impact on mitochondrial ATP production within muscle (Li and Suave, 2015). Canto et al. (2012) and Zang et al. (2016) demonstrated NR efficacy on non-invasive endurance analysis in addition to molecular analysis. Both studies performed endurance tests between NR dosed which resulted in 28 and 31% increases in running distance compared to their control diet counterparts (Canto et al., 2012; Zang et al., 2016). Therefore, the objective of this study was to determine the daily dietary NR dose required to maximize the amount of NAD+ produced in 3 ambulatory muscles and evaluating the effect of increasing dietary NR dose on delaying the onset of muscle fatigue.

# CHAPTER 2

# REVIEW OF LITERATURE

## Muscle Fatigue

### *Muscle Structure and Contraction*

Muscle fatigue has many definitions including decrease in muscle performance capacity (Asmussen et al., 1979), acute impairment of performance followed by the inability to produce effort to complete an action (Enoka and Stuart, 1992), and in the swine industry, when pigs are unable to walk or keep up, but show no signs of injury or trauma (Ritter et al., 2009). The biology behind muscle fatigue begins with three layers of connective tissue known as mysia. Mysia provides structure by enclosing the muscle as a whole. Each muscle is wrapped in epimysium which connects muscle to the bone in order to transfer force for the muscle contraction to the bone. Inside each muscle, individual muscle fibers are grouped into fascicles, or bundles, which are held together and separated from others by perimysium connective tissue. Within the perimysium, each fiber is surrounded by a layer of connective tissue known as endomysium.

Individual muscle fiber structure begins with surrounding sarcolemma plasma membrane filled with sarcoplasm. Occasionally, along the length of muscle fibers, in-folding of sarcolemma result in networks of tubules called transverse tubules, or T-tubules. Myofibrils are found within the sarcolemma and run the entire fiber length. These functional units of the muscle are made of thin filaments, actin, and thick filaments, myosin. The arrangements of these two filaments result in the striated appearance of muscle fibers. The areas of varying densities to result in the striation, are given different names. The singly refractive, light band was termed I band for being described as isotropic; whereas, in comparison, the doubly refractive, broad, dark band is termed

the A band. Other features of myofibril exist as zones including the H zone and M line. Only myosin filaments are found within the H zone, actin filaments are found within the I band, and both overlap within the A band. The endoplasmic reticulum within each muscle fiber is the sarcoplasmic reticulum (**SR**), and its job is to store, release, and retrieve calcium ions (Aberle et al., 2001).

Six major proteins account for 90% of total myofibrillar proteins including myosin, actin, titin, tropomyosin, troponin, and nebulin. Proteins are classified according to contractile, regulatory, or cytoskeleton properties. Regulatory proteins act as actin-myosin interaction regulators during muscle contraction. Cytoskeleton proteins act as myofilament alignment templates during myofibril and sarcolemma formation. Myosin and actin are classified as contractile, tropomyosin and troponin as regulatory, and titin and nebulin as cytoskeleton (Aberle et al., 2001).

Muscle contractions are initiated through stimuli arrival at the sarcolemma. Under normal resting conditions, an electrical potential exists between inside and outside of cells. This electrical potential is maintained by a positive and negative ion balance inside and outside the cell, respectively. Extracellular fluid contains great amounts of sodium (**Na**+) and chloride (**Cl**-) ions, while intracellular fluid contains excess amounts of potassium (**K**+) ions. Sodium active transport out of the cell in conjunction with K+ transport into the cell maintain ideal concentration gradients. Energy required to pump both ions across the membrane to return Na+ and K+ to resting rates and maintain ionic balance is referred to as adenosine triphosphate (**ATP**; Aberle et al., 2001).

Nerve and muscle fibers both exhibit membrane potentials similar to other cells; however, they are able to transmit an electrical impulse, or action potential, along their

membrane surfaces. Action potentials signal depolarization, or reversal of electrical charges within the cell's membrane, which is achieved by a dramatic increase in membrane permeability resulting in $Na^+$ rushing into the cell. As action potentials move along muscle fibers through T-tubules, it is responsible for $Ca^{2+}$ release from SR (Aberle et al., 2001).

Calcium concentrations initiate the contractile process by signaling tropomyosin and troponin. Troponin, once activated, interacts with tropomyosin to unblock active sites between the actin and myosin filaments allowing for cross-bridge formation. Contraction is initiated with an increase in free $Ca^{2+}$. Cross-bridge formation produce enough force to slide actin filaments 10 nm. Throughout contraction, cross-bridge formation and breaking is required to generate force (Aberle et al., 2001). The myosin head attaches to actin while ADP and inorganic phosphate (P*i*) are bound to the myosin of the sarcomere. Inorganic phosphate is then released when the myosin head attaches to actin causing an even stronger connection between actin causing a forward pulling motion known as the power stroke (Betts et al., 2017).

A-band width is maintained through all phases of contraction; however, I band and H zone width change. Widths are greater while muscle is stretched and decrease as muscle is shortened. During contraction, myosin ATPase is activated and ATP is hydrolyzed in order to provide energy to maintain contractions. As long as ATP is readily available, it can attach to myosin and muscle contraction can occur. Once ATP decreases or is exhausted, fatigue is experienced. After contractions, actin-myosin crossbridge formations are terminated, Mg2+ complexes reform with ATP, and Ca2+ is returned to SR, all resulting in sarcomeres and muscles returning to resting states (Aberle et al., 2001).

### *Muscle Fibers and Fatigue*

A major influencer of muscle fatigue onset is the muscle fiber type composition of the muscle. There are many divisions between muscle fiber type which include contraction speeds and metabolism (Bottinelli and Reggiani, 2000). Muscles have historically been classified based off their fresh color intensity, which is differentiated by the red and white fibers the muscles contain. It should be noted, muscles rarely contain solely red or white fibers, but are composed of a red and white fiber mixture. Combined, contraction speed and metabolism allow for four adult isoforms in skeletal muscles, including: Type I, IIA, IIX, and IIB. In terms of contraction speed, type I fibers are slower contracting fibers while type II are faster contracting fibers although the speed only differs by fractions of a second (Aberle et al., 2001). Muscle fiber energy requirements, or metabolism, often correlates with the rate of cross-bridge cycling, or contraction speed. As slow-twitch fibers, type I, require the lowest level of energy and can sustain its energy through long period of times, intermediate and fast-twitch fibers, type IIA, IIX/B, respectively, have a greater energy requirement and burn through energy easier (Julien et al., 2018).

Classification in terms of contraction speed has relevance to fatigue in that faster contracting muscle fibers will consume ATP at a much greater rate than slower contracting muscle fibers. White muscle fibers have a phasic mode of action during contraction, meaning they contract rapidly and in short bursts; whereas, red fibers have a tonic mode of action where they contract more slowly for a longer time (Aberle et al., 2001). Thus, fatigue is resisted to a greater extent when the ATP consumption is decreased during a more prolonged cross-bridge cycle. The other major ATP-consuming protein in muscles is the sarcoplasmic reticulum $Ca^{2+}$ pumps. There are two different forms of these pumps, sarcoendoplasmic reticulum calcium

ATPase (**SERCA**) 1 and SERCA 2. Fast, type II, muscle fibers tend to contain SERCA1 pumps while SERCA2 pumps are found in slower, type I, muscle fibers (Lytton et al., 1992).

Myoglobin content is responsible for the difference in fresh meat color but is also responsible for oxygen storage involved in oxidative metabolism (Aberle et al., 2001). The oxidative capacity of the different muscle fiber types also relates to fatigue resistibility. This oxidative capacity is achieved through varying degree of myoglobin, as mentioned, but also of mitochondrial concentration and size. Slow, red fiber types typically possess a much larger concentration of mitochondria matched with greater size; thus, they also have a much greater oxidative capacity when compared to fast, white muscle fibers (Essen et al., 1975).

***Theories of Muscle Fatigue***

Enoka and Stuart (1992) theorized four different hypotheses as to the mechanisms of fatigue. The first theory suggested, task dependency, was characterized as fatigue being a consequence of not only one mechanism, but rather a list of mechanisms. Mechanisms which might stimulate fatigue include motivation of the subject performing the fatiguing activity, activity intensity and duration, speed of muscle contractions, and extent to which the activity was sustained. The authors suggested combination of these mechanisms exacerbated muscle fatigue. The second theory proposed was the relationship between force and fatigability. This theory was characterized as neuromuscular system activation at any intensity will eventually lead to fatigue. The major concept to understand from this theory is as force exerted on a task increased, the more rapidly muscle fatigued and the greater fatigue experienced.

The third theory gave rise to the term muscle wisdom. Muscle wisdom was the optimization of force which ensured activation of fatiguing muscles by the central nervous system. This is accomplished by declined force, declined relaxation rate, and declined motor

neuron discharge. The fourth and final theory is the sense of effort theory. Subjects must understand there is necessary minimum effort associated with tasks, and if subject is not willing to endure effort, fatigue will arise more rapidly and forcefully.

Fatigue might be resisted by many different methods; one popular theory involves the varying muscle fibers activated by the central nervous system (**CNS**). Less fatigable muscle fibers may be utilized for a much lower work load; whereas, the greatest power and easily fatigable units are recruited during much more demanding tasks (Bigland-Ritchie et al., 1986). This will not prevent fatigue, but it will allow the subject to carry on with the task without being easily fatigued. As well as CNS monitoring motor units, action potential firing reduction might help in resisting fatigue. If motor units are stimulated at a greater than optimal firing rate, muscle fibers are much more likely to quickly fatigue, whereas if action potential excitation is kept to a minimum, fatigue may be delayed (Jones et al., 1979).

## Transportation and Stress Factors

### *Fatigued Pig Syndrome*

The term "Fatigued Pig Syndrome" is used to describe a pig that arrives to the abattoir showing no physical signs of disease, trauma, or injury but refuses to walk, move, or keep up with its other trailer mates, despite being able to stand on their own (Ritter et al., 2005; Doonan et al., 2014). These pigs have also been described as non-ambulatory, but non-injured (Fitzgerald et al., 2009). These pigs show physical symptoms which allow for identification such as open-mouth breathing, splotchy skin discoloration, shoulder and ham muscle tremors, and abnormal vocalizations (Anderson et al., 2002).

These pigs are in a state of metabolic acidosis with greater blood lactate, decreased blood pH, and decreased bicarbonate values than their non-fatigued counterparts (Edwards et al.,

2011). In reaction to stress, muscle metabolism becomes deranged causing susceptible pigs shift their energy utilization from a normal aerobic pathway to anaerobic pathway; therefore, leading to increased body heat production. As body heat continues to rise, homeostasis in the body fails, muscles slowly become unable to function, and they develop an irreversible rigidity prior to death (Smith et al., 1976).

***Transport Losses***

When pigs arrive to a slaughter facility they are classified based on their physical appearance and movement. Besides pigs who arrive healthy and unproblematic, there are five other classifications pigs can be placed in. The first group are dead-on arrival (**DOA**) pigs which arrive to the facility dead on the trailer. The second group is euthanized on arrival (**EOA**). These pigs have a severe injury which requires immediate euthanasia or physically cannot walk off the truck. The third group is dead-in-pen (**DIP**) which are pigs that die during lairage, or resting period between arriving to the abattoir and slaughter. The fourth group are injured pigs, which have a noticeable structural injury when arriving to the facility, but do not require euthanasia. The fifth group are non-ambulatory, non-injured pigs which are labeled as fatigued (Ritter et al., 2020).

Ritter et al. (2009) summarized 23 trials' reports of dead and non-ambulatory pig occurrences after transport in the United States in 2006. The review reported average transport losses for dead and non-ambulatory pigs were 0.22 and 0.44%, respectively. Between 2012 and 2015, those averages have increased as reported in an industry survey representing 310 million market weight pigs across 20 U.S. abattoirs, DOA, EOA, DIP, and fatigued pigs accounted for 0.15, 0.05, 0.05, and 0.63% loss of recorded pigs, respectively. The annual cost based off these values lost to the pork industry is $89 million (Ritter et al., 2020). It needs to be understood that

transportation losses are a multifactorial issue including injury, fatigue, and death (Ritter et al., 2009). The contributors most evaluated are related to stress in swine both before and after they arrive to the slaughter abattoir.

***Stress Factors***

Many studies were performed regarding evaluation of different stress factors which may contribute to swine industry transport loss occurrence. A study in London performed over 4- and 12-year periods, reported on the relationship between recorded losses both during and after transport and the effect daily weather had. There was a decrease in transport deaths when temperatures were below 10°C but greater when temperatures were above 18°C. When total deaths were measured from January to July the death rate was 12% greater when compared to the death rates from the months of August to December. When evaluating the death rates by individual months, notoriously hotter, summer months—June, July, and August—had a combined 85% increase in DOA, DIP, and total deaths when compared to notoriously cooler months—December, January, February (Smith and Allen, 1976). Fitzgerald et al. (2009) found increasing trailer density by 50 and 100 $kg/m^2$ increased total deaths per trailer by 0.53 and 0.74%, respectfully. There was also an 755% increase in trailer loss for maximum trailer densities compared to minimum densities.

Feed withdrawal time periods, heavy target market weight of pigs, the overall facility design and pre-sorting procedures, and loading and unloading procedures might also serve to encourage transport losses (Doonan et al., 2014). Feed withdrawal period is defined as the period of time when pigs are denied feed prior to slaughter. Feed withdrawal is critical in reducing in transit loss, improving animal well-being, pork safety, and pork quality. Pigs with full stomachs are more likely to be subjected to transport death along with motion sickness and vomiting.

During the slaughter process they are also more prone to intestinal rupture, gut spilling, or rectal leakage which can contaminate the carcass. Withdrawal times of 12-18 h prior reduces in-transit loss while enhancing pork quality and animal welfare (Bidner and McKeith, 1998; Murray et al., 2001).

Gesing et al. (2010) investigated pre-sorting effects on pigs' stress responses at loading and unloading. Sorting was accomplished by dividing pens into smaller sized pens using swing gates. Pigs were sorted into smaller pens based on time it took to reach market weight with fastest growing pigs in first group, intermediate growth pigs in second, and remaining pigs in final and largest group. Pigs were sorted into their corresponding growth groups 18 h prior to loading. By separating test subjects into two groups: one where no sorting was done prior to loading and one where sorting was done prior to trailer loading, researchers were able to conclude stress responses during loading were significantly reduced through blood analysis and took less time for loading for the group who were pre-sorted prior to loading.

A study performed by Ritter et al. (2009) evaluated a combination of varying handling intensities, transportation floor space, and distance moved to understand effects of different stressors on finishing pigs' responses. Gentle handling consisted of moving pigs at their own pace with a sorting board and paddle whereas aggressively handled pigs were moved utilizing an electric prod and sorting board. Transportation floor space were evaluated by placing eight pigs in a trailer compartment of either 0.39 or 0.49 $m^2$/pig. Pigs were then either moved 25 or 125 m through a handling course prior to loading. The study found pigs with restricted floor spacing experienced more physical stress and muscle damage as well as pigs who were handled aggressively and those who were handled for the longer distance (Ritter et al., 2009).

Regular handling and moving of the pigs prior to loading and transporting to slaughter plants allows the pigs to become familiarized with the action and overall reduces psychological stress (Doonan et al., 2014). Ramps also tend to cause trauma and stress to pigs, so ramp usage should be avoided, or, if they are necessary, should not exceed a 20° incline (Warriss et al., 1991). A cumulative effect can be observed when there is a combination of stressors present to pigs at or prior to the abattoir. Removing or reducing even one stressor will show favorable improvements in overall transportation, or total, losses. Action is being taken at slaughter houses through regulation establishment, but they must be taken on-farm as well to efficiently minimize stress factors which result in transport losses at the abattoir.

**Electromyography**

Surface electromyograph (**EMG**) is a useful, non-invasive tool regarding assessment of muscle load and muscle fatigue (Bartuzi et al., 2007). This muscle evaluation form of was used in order to receive information regarding time, duration, and muscle contraction phasic relationship. It has also been useful in diagnosis of neuromuscular diseases (Ralston, 1961). In biomechanics, EMG has three main uses. The first being an indicator of muscle activation initiation, second being supplying researchers with a relationship of force produced by a muscle, and finally, it is used as a muscle fatigue indicator as it occurs in live time. As a muscle fatigues, it is accompanied by a decrease in muscle electrical activity through the loss of recruited and active muscle fibers (Dimitrova and Dimitrov, 2003).

There are multiple influencers of EMG including causative, intermediate, and deterministic factors. Causative influencers include elements that have a basic or elemental effect on the EMG signal. These influencers can be broken down into extrinsic factors, associated with physical electrode structure and placement on the skin and intrinsic factors, associated with

physiological, anatomical, and muscle biochemical characteristics. Intermediate influencers represent physical and physiological outcomes resulting from one or more causative factors. The deterministic influencers have a direct bearing on the EMG signal information. This is influenced by active motor units, motor firing rate, number of detected motor units, and motor unit recruitment stability (De Luca, 1997).

The main, and most accurate, muscle fatigue indicators generated by EMG data include root mean square (**RMS**), mean power frequency (**MPF**), and median power frequency (**MdPF**). Root mean square, defined as assessment of muscle load in time domain on the basis of amplitude (Bartuzi et al., 2007), serves as a means of evaluating muscle fiber recruitment by comparing active muscle fibers to the number utilized during rest (Noel et al., 2016). In regard to muscle fatigue, as subjects fatigue there will be an increase in RMS. Both MPF and MdPF are defined as a signal power spectrum parameter. These two measurements record the velocity at which the motor unit action potential moves along the muscle fibers during contraction (Noel et al., 2016). As subjects fatigue, both MPF and MdPF will decrease.

Electromyography has been utilized in research in not only humans (Bartuzi et al., 2007) but also horse, sheep, and swine studies (Robert et al., 2001; Zsoldos et al., 2010; Cockram et al., 2012; Noel et al., 2016). Noel et al. (2016) evaluated Ractopamine HCl (**RAC**) effect on barrow muscle fatigability. Researchers analyzed muscle fiber activity of deltoideus, triceps brachii lateral head, tensor facia latae, and semitendinosus muscles. Results showed RAC fed subjects had no change in MdP within all muscle groups. End-point RMS values indicated RAC fed groups fatigued more quickly than control (**CON**) fed barrows. Results showed a decreased end-point RMS in the semitendinosus muscle which was compensated by an increase in end-point RMS of the tensor fasciae latae to allow for continued movement during the performance test.

Robert et al. (2001) and Zsoldos et al. (2010) studied the changes in abdominal muscle activity during a walk and trot of horses. Electrodes were place on the gluteus medius, tensor fasciae latae, rectus abdominis, and oblique external abdominal muscles. Horses were walked then trotted around a track while EMG data were collected. Results showed as the horses were trotted, EMG values were significantly increased in all horses in all muscle locations compared to walking. Conclusions stated the higher activity of trotting at increased speeds resulted in greater EMG activity and thus a higher workload to the muscles measured. These studies show the tremendous impact EMG can have on determining muscle activity and fatigue and the usefulness across different species.

## Blood Parameters Associated with Fatigue

### *Lactate*

Lactate is a by-product of anaerobic glycolysis, or the breakdown of glycogen and glucose. In summation, as the body metabolizes glycogen or glucose, pyruvate is formed. If by this point, there is a surplus of energy in the system, pyruvate will be converted to Acetyl Co-A. The alternative states with a lack of energy, or similarly, during exercise where energy is being utilized, pyruvate is converted to lactate (Rogatzki et al., 2015; Nalbandian and Masaki, 2016). Fatigue can be determined through blood parameter analysis. One of the most significant fatigue indicators is shown through lactate accumulation post short-term exercise (Sahlin, 2012). In a study evaluating lactate levels during a soccer match, players experiencing the greatest lactate levels were those performing the most work throughout the game (Bangsbo et al., 2007). Another study performed on human subjects showed treatment groups subjected to high intensity exercise had a greater blood lactate concentration than those treatment groups allowed to rest (Nozaki et al., 2009). In equine, lactate has been shown to directly correlate to amount of work,

as horses subjected to a more intense exercise protocol produced an increase in blood lactate compared to those horses given a more lax protocol (Daden et al., 2019). Similar studies performed by Thomson et al. (2015) and Hagemaier et al. (2017) in beef cattle produced results similar to horses; however, cattle were evaluated after demonstrating non-ambulatory, non-injured behavior which researchers attribute to handling stress. In cattle demonstrating fatigued behaviors, lactate levels were increased from the normal range which was attributed to increased stress exposure and responses.

***Cortisol***

Cortisol, a steroid hormone, was referred to as the body's physiological response to stress (Duran-Pinedo et al., 2018). Cortisol is available and able to affect almost all organ systems in the body, including the musculoskeletal system. In times of stress, the sympathetic nervous system is activated and responds by signaling the adrenal glands to release catecholamines. Catecholamines work to activate the hypothalamic-pituitary-adrenal axis which signals the adrenal cortex to release cortisol into the body (Krumrych et al., 2017.Thau et al., 2021). In response to exercise, the body reacts similarly as it would to stress. Nozaki et al. (2009) evaluated metabolic differences between human subjects exposed to intense exercise programs and those exposed to no exercise program. Similar to lactate, the greater intensity treatment group resulted in increased blood cortisol concentrations compared to the rested group. In a study by Echternkamp (1984), beef cattle allowed to acclimated to varying physical restraint means and frequent blood collection had decreased cortisol concentrations compared to a group of beef cattle not allowed time to acclimated prior to sample collection. These results indicate the non-acclimated group experienced a greater level of stress than the group familiar with the procedures. Sheep have also been evaluated for metabolic response of stress by subjecting all

sheep to intense treadmill exercise; however, sheep were separated into two groups. The first was allowed to run next to another sheep to maintain their need for companionship, but the second ran alone. Both groups showed increased blood cortisol post-exercise, but the group made to run alone had on average a much greater increase in cortisol levels (Cockram et al., 2012). These results demonstrated cortisol's reaction to both exercise as well as stress.

***Creatine Kinase***

Creatine kinase is introduced as a catalyst for the body's energy production system, as it allows the phosphate to be cleaved off phosphocreatine and donated to ADP, in order for it to produce ATP, or energy for the system (Baird et al., 2012). Creatine kinase has been defined as an indirect marker of muscle damage, which can be useful in evaluating fatigue in subjects after exercise as it is most reliable at showing the greatest variability between subjects exposed to varying exercise intensities (Hody et al., 2014; Kim and Lee, 2015; Hagstrom and Shorter, 2018). Nozaki et al. (2009) subjected volunteers to a fatigue-inducing biking task for 2 h, allowed a 20 min rest period, and 2 h bike task again. Blood samples were taken after exercise and compared to subjects allowed to relax the entire period. Participants exposed to the exercise protocol had greater creatine kinase values than relaxed participants. A study evaluating sheep creatine kinase response to treadmill exercise showed similar trends where after exercise, sheep exposed to a harsher exercise regimen had a greater increase in creatine kinase levels post-exercise (Cockram et al., 2012). A handling intensity study on cattle showed cattle experiencing greater intensity handling measures had an increased creatine kinase response in comparison to the cattle exposed to a less intense handling protocol (Hagenmaier et al., 2017). Daden et al. (2019) took blood samples from horses before and after bursts of exercise. In all horses,

immediately following exercise, creatine kinase was greater than pre-exercise samples and after an allotted rest period.

***Glucose, Insulin, and Glycogen***

Glucose is universally a major source of energy for every organism in the world (Richter et al., 2001a; Hantzidiamantis and Lappin, 2020). It is provided to organisms through many isometric forms including: mono-, di-, and polysaccharides. Excess glucose is stored in the body as glycogen for future usage during fasting states. Both glycogen and glucose are regulated by the hormone, insulin. Insulin is produced in the pancreas by β-cells and is used when transporting glucose in and out of cells. Without the help of insulin, it would be difficult, and near impossible, for glucose or glycogen to be converted to pyruvate through glycolysis. As with glucose, insulin is greater during energy rich states, as it is released with the job to move glucose where it is needed throughout the body (Berg et al., 2002; Hantzidiamantis and Lappin, 2020).

Physical exercise induces a rapid increase in the rate of glucose uptake in the contracting skeletal muscles. Previous literature showed glucose uptake was greater during exercise within humans during glycogen depleted states rather than glycogen rich states (Richter et al., 2001). Nozaki et al. (2009), showed a drastic decrease in glucose levels after exercise in humans. Horses showed similar trends where prior to training, glucose levels were increased prior to activity and decreased after activity. When insulin was evaluated in these same horses, insulin had the opposite reaction where levels increased after activity (Bonelli et al., 2017).

Glycogen is a readily mobilized storage form of glucose; therefore, glycogen is a good source of energy for sudden, strenuous activity (Berg et al., 2002). It is not evenly distributed throughout skeletal muscle fibers, but rather is localized in pools. One major pool of glycogen can be found within the myofibrils associated with the excitation-contraction coupling reaction

and calcium release from the sarcoplasmic reticulum. Due to the proximity and relationship to SR, glycogen is essential in order for ATP to be rapidly produced. As glycogen acts as an energy source during exercise, levels are decreased after exercise (Hespel and Richter, 1990; Jensen et al., 1997; Jensen et al., 2005).

Hespel and Richter (1990) studied the effects resting and contracting skeletal muscles would have on glucose and glycogen levels of rats. As rats were exercised, muscle glucose uptake increased 69% from resting state to 15 min of exercise. The glucose uptake changes observed were negatively correlated to the muscle glycogen concentration, as exercise was experienced, glycogen concentration decreased around 40%. Similarly, Jensen et al. (1997) evaluated rat skeletal muscle glucose uptake and glycogen concentration, and found a negative correlation whereas glucose uptake increased, glycogen concentration decreased.

**NAD+ and NADH on Muscle Fatigue**

Mitochondrial ATP production requires the coenzyme nicotinamide adenine dinucleotide (NAD+). This coenzyme is involved in cellular energy metabolism and energy production through its involvement in degradation of carbohydrates, fats, proteins, and alcohol (Bogan and Brenner, 2008). Nicotinamide adenine dinucleotide and its reduced form, NADH, are required for metabolic reactions to occur within mitochondria. During carbohydrate metabolism, glycolysis, glucose is converted to pyruvate through glyceraldehyde-3-phosphate dehydrogenase requiring two NAD+ per glucose to form pyruvate. Pyruvate and NADH produced by glycolysis are then transported from the cell's cytoplasm to the mitochondrial matrix. There they provide reducing equivalents for the citric acid cycle (**TCA**) and the electron transport chain (**ETC**), both of which are responsible for ATP production. Two electrons and protons from varying substrates are donated to NAD+ at multiple TCA steps, which are reduced to NADH. Mitochondrial

NADH is transported further through the ETC by the malate-aspartate and glycerol-3-phosphate redox shuttles (Li and Sauve, 2015).

At the ETC, NADH is oxidized by donating its electrons to Complex I. Complex I is responsible for transporting the donated electrons to Complex II, III, and IV of the ETC. This flow of electrons throughout the ETC is also accompanied by the pumping of protons by Complexes I, III, and IV. In the end, oxygen is reduced to water through the electron donation passed between complexes and the protons are pumped through Complex V, otherwise known as ATP synthase (Stein and Imai, 2012). Both TCA and ETC require an optimal NAD:NADH ratio for efficient mitochondrial metabolism. Levels of NAD+ are limiting which makes availability critical for proper mitochondrial function. Biosynthesis of NAD+ is modulated in response to nutritional and environmental stimuli. Exercise is a large effector of NAD+ and NADH production (Stein and Imai, 2012; White and Schenk, 2012). During muscle contraction, NADH levels decrease as they are being utilized for ATP production to maintain energy and NAD+ levels increase (Chance and Connelley, 1957).

Many past studies evaluated the inverse relationship between NADH and NAD+ through fluorescence-based methods in both rats and canines. Jobsis and Stainsby (1968) demonstrated within canine gastrocnemius-plantaris and gracilis muscle groups, NAD+ levels increased after exercise exposure, which was also accompanied by decreased NADH levels. A similar study by Duboc et al. (1988), was performed using mouse soleus and extensor digitorum longus muscles. Within these muscles, NAD+ increased and NADH decreased following exercise. A method known as the metabolite indicator method was introduced to measure concentrations of specific cytoplasmic and mitochondrial redox couples in order to accurately quantify NAD+ and NADH concentrations (White and Schneck, 2012). Rats were evaluated pre and post- exercise and

NAD+ concentrations increased while NADH concentrations decreased (Edington and McCafferty, 1973; Wendt and Chapman, 1976). Rats were again evaluated but the method of exercise training was altered to swimming training. Regardless of the shift in exercise form, the same findings were seen; NADH levels decreased and NAD+ levels increased following swimming exposure (Canto et al., 2010; Koltai et al., 2010).

**Nicotinamide Riboside**

Pellagra is described as a deficiency of niacin and tryptophan. This deficiency was extremely common more than a century ago and resulted in muscle and liver NAD+ deficiencies. Nicotinamide adenosine dinucleotide is involved in energy producing catabolic reactions and deficiencies result in diarrhea, dermatitis, dementia, and death. Common treatments for pellagra included fresh milk, eggs, and meat which had natural sources of niacin and tryptophan. In early 1900, researchers discovered nicotinamide fraction (**NAM**) and nicotinic acid (**NA**) had an anti-pellagragenic effect on malnourished dogs (Bieganowski and Brenner, 2004). Currently research has been done to understand the effects NAM, NA, and tryptophan have on pellagra and NAD+ deficiencies.

Tryptophan, NA, and NAM are all proven precursors to NAD+; however, each uses a different mechanism and has a different result. Tryptophan utilizes an 8-step de novo pathway in order to create NAD+. Nicotinic acid and NAM are considered salvage precursors that require 2- and 3-steps, respectively, to rebuild NAD+ (Bogan and Brenner, 2008; White and Schnek, 2012). Recently there has been a fourth alternative NAD+ precursor, nicotinamide riboside (**NR**) discovered by researchers. Nicotinamide riboside uses a 2 or 3-step salvage pathway in order to create NAD+, resulting in NR being a more efficient creator of NAD+ compared with tryptophan, NA, and NAM (Belenky et al., 2006).

Nicotinamide riboside, a naturally occurring vitamin B3 analogue, has been claimed to delay aging, improve brain function, and maintain intracellular NAD+ concentrations and NAD+ dependent activities. There have been many studies testing these claims. In a study by Conze et al. (2019) NAD+ production and its relation to NR dosage was evaluated. Blood and urine were evaluated for NAD+ concentrations throughout the 8-week study with subjects separated into 4 treatment groups all with varying NR dosages, 0, 100, 300, and 1000 mg NR. All subjects began d 0 with similar NAD+ concentrations. By d 56, all treatment groups receiving NR had greater NAD+ concentrations compared with placebo group. Conze et al. (2019) confirmed NR effects through evaluation of muscle nicotinamide (**NAM**), methylated muscle nicotinamide (**MeNAM**), and N1-methyl-2pyridone-5-carboxamide (**Me2PY**) which are all biomarkers of NAD+ production. Each value increased between d 0 and 56 of supplementation with greatest increased seen in treatment groups given largest NR dosage.

Another study evaluated mitochondrial quality within muscle and brown adipose tissue (**BAT**) in deletor mice and wild type mice. Cristae count and mitochondrial DNA quantity increased in both tissues within both mice groups supplemented NR. These data demonstrate a positive effect of NR on mitochondrial quality which may directly impact NAD+ production ability (Khan et al., 2014). A similar study by Canto et al. (2012) evaluated NAD+ concentration within 5 tissues: brain, muscle, liver, BAT, and white adipose tissue (**WAT**) in two mice treatment groups. The treatment group fed an increased fat control diet displayed, on average, a 32% decrease in NAD+ concentrations within all tissues when compared to the treatment group fed a high fat NR supplemented diet. Both studies demonstrated NR potential to increase NAD+ concentration among different tissue groups independent of diet type and can rescue metabolites of predisposed mitochondrial diagnosed patients.

Canto et al. (2012) established NR efficacy in NAD+ concentrations but evaluated endurance in mice fed both control and supplemented groups, as well. Mice fed 400 mg • kg body weight$^{-1}$ • d$^{-1}$ NR diet demonstrated increased endurance compared to control group through an 28% increase in distance ran during a performance test. Another endurance-based NR study performed by Zang et al. (2016) evaluated running duration, running distance, and grip strength in older mice fed a control diet versus an NR supplemented diet. In all parameters measured, the aged mice fed the NR diet showed an averaged 36% increase in performance than their control counterparts; however, there was no difference reported in young mice between the two treatment groups performances. These two studies allow conclusions regarding NR positive effect on endurance and potential for fatigue resistance in supplemented subjects.

# CHAPTER 3

# EFFECTS OF VITAMIN B3 DOSE ON MARKET BARROW FATIGUE ONSET AND GAIT[1]

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## ABSTRACT

The objective of this study was to determine the daily dietary nicotinamide riboside (**NR**) dose required to maximize delaying the onset of subjective fatigue. Barrows ($N$ = 100) were assigned to 1 of 5 treatments: a conventional swine finishing diet containing 0 (**CON**), 15 (**15NR**), 30 (**30NR**), 45 (**45NR**) mg•kg body weight$^{-1}$•d$^{-1}$ NR, and 0 NR barrows supplemented 45 mg•kg body weight$^{-1}$•d$^{-1}$daily in Karo Syrup (**DRE**) for periods 1 to 3 or raw cookie dough for periods 4 and 5. All treatments were administered the final 11 days of feeding. On d 0, 5, 10 of supplementation, muscle biopsies were collected from the biceps femoris (**BF**), tensor fasciae latae (**TFL**), and semitendinosus (**ST**) muscles for NAD+ analysis. On d 10 of supplementation, pigs were snared and bled for pre-fatigue blood analysis. After 10 d on feed, barrows were individually moved around a track at 1.09 m/s until subjectively exhausted. Wireless electromyography (**EMG**) sensors were affixed to the BF, TFL, and ST to measure active muscle fiber recruitment in real time. Barrows were walked on pressure mat before and after fatigue for gait analysis. Immediately following fatigue, pigs were bled for post-fatigue blood analysis. After harvest, samples of each muscle were collected for fiber type and succinate dehydrogenase (**SDH**) analysis. Speed, time, and distance to exhaustion did not differ between treatment ($P$ > 0.17). Electromyography showed an overall decrease ($P$ < 0.01) over time with root mean square (**RMS**) values being greatest ($P$ < 0.01) in ST muscles indicating a loss of active muscle fiber recruitment. Gait4 analysis did not differ between treatments ($P$ > 0.30), but was different for all parameters, except step length ($P$ = 0.67), pre- versus post- exhaustion ($P$ < 0.05). Serum lactate and creatine kinase (**CK**) had greater values in CON barrows compared to all other treatments ($P$ < 0.03). Barrows in the 45NR treatment had more type IIX fibers type than CON, 15NR, and 30

NR barrows ($P < 0.05$). Overall, NR demonstrates potential in being a useful tool in fatigue prevention with reduction of blood fatigue metabolites, numerically greater run time and duration in DRE barrows, and increase in transitional fiber types with potential for greater improvements through increased supplementation period due to 2-week period for proper muscle fiber type turn around.

Key words: *fatigue, muscle fiber, nicotinamide riboside, barrow*

## ABBREVIATIONS

**15NR**; 15 mg•kg body weight$^{-1}$•d$^{-1}$ feed treatment

**30NR**; 30 mg•kg body weight$^{-1}$•d$^{-1}$ feed treatment

**45NR**; 45 mg•kg body weight$^{-1}$•d$^{-1}$ feed treatment

**BF**; biceps femoris

**CK**; creatine kinase

**CON**; 0 mg•kg body weight$^{-1}$•d$^{-1}$ feed treatment

**DRE**; 45 mg•kg body weight$^{-1}$•d$^{-1}$ administered by drench or cookie dough

**EMG**; electromyography

**LARU**; Large Animal Research Unit

**MQ**; milli-Q

**MSTC**; Meat Science and Technology Center

**NAD+**; nicotinamide adenine dinucleotide

**NADH**; nicotinamide adenine dinucleotide + hydrogen

**NR**; nicotinamide riboside

**OCT**; optimal cutting temperature

**PBS**; phosphate buffer solution

**PO**; post-exhaustion

**PRE**; pre-exhaustion

**SDH**; succinate dehydrogenase

**ST**; semitendinosus

**TFL**; tensor fasciae latae

## INTRODUCTION

Fatigued pig syndrome can be used to characterize non-deceased, non-ambulatory, and physically, non-injured pigs arriving at the abattoir demonstrating typical signs of fatigue including open-mouth breathing, muscle tremors, and increased reddening of the skin (Benjamin, 2005; Ritter et al., 2009). Typically, fatigued pigs recover during a 2 to 3 h lairage period after transportation to the abattoir; however, many pigs die before they recover (Hamilton et al., 2004; Ritter et al., 2005). Ritter et al. (2009; 2020) summarized 23 field studies between 2000 and 2007 and reported fatigued-pig transport loss percentage between 2009 and 2012. In 2009, 0.44% of pigs were classified as fatigued while that number increased to 0.66% by 2020 indicating this is a growing issue. These studies estimated dead and non-ambulatory pigs cost the U.S. swine industry $46 million (UDS) in 2009, with values increasing to $88 million in 2020. Numerous environmental factors including season, trailer density, sorting methods, and feed withdrawal times were studied to understand fatigue in response to stress sources in pigs (Smith et al., 1976; Fitzgerald et al., 2009; Gesing et al., 2010; Doonan et al., 2014); however, biological stress indicators and mechanisms related to the muscle fatigue portion of pig fatigability have limited research available.

Internal substrates related to muscle fatigue include pyridine nucleotides, nicotinamide adenine dinucleotide (**NAD**+) and nicotinamide adenine dinucleotide + hydrogen (**NADH**). These nucleotides are coenzymes in muscle mitochondria which provide oxidoreductive power to the electron transport chain which is responsible for ATP production (Bogan and Brenner, 2008; Egan and Zierth, 2013; Li and Suave, 2015). Glycolysis production of NADH is utilized in the electron transport chain for both NADH and ATP production. In past studies, increased NAD+ levels accompanied by decreased NADH levels were observed following exercise

training in both rats and canines (Jobsis and Stainsby, 1968; Duboc et al., 1988). The levels of NAD+ in resting muscles are seen to be greatest in mitochondria dense fibers such as type I muscle fibers (Graham et al., 1978; Ren et al., 1988), which could indicate fiber type composition influences NAD+ levels. With the influence mitochondria concentration has on NAD+ and NADH levels, manipulation of mitochondrial biogenesis or efficiency could increase potential ATP production and fatigue resistance.

Supplemental nicotinamide riboside (**NR**), a naturally occurring vitamin B3 analogue, was studied in mice and humans and showed NR positively affected varying muscle groups' NAD+ concentrations (Canto et al., 2012; Zhang et al., 2016; Airhart et al., 2017; Conze et al., 2019). Khan et al. (2014) demonstrated NR supplemented mice, predisposed to mitochondrial myopathy, resulted in increased mitochondrial DNA, indicating increased energy production potential through mitochondrial biogenesis. These results are ideal as NAD+ has a direct impact on mitochondrial ATP production within muscle (Li and Suave, 2015). Canto et al. (2012) and Zang et al. (2016) demonstrated NR dosed mice had increased running distance by 28 and 31%, respectively. Therefore, the objective of this study was to determine the daily dietary NR dose required to maximize the amount of NAD+ produced in 3 ambulatory muscles and evaluating the effect of increasing dietary NR dose on delaying the onset of muscle fatigue.

## MATERIALS AND METHODS

The University of Georgia Institutional Animal Care and Use Committee approved the protocol used in this experiment AUP# A2020 03-004-R2.

### *Live Animal Management*

Finishing barrows ($N = 100$; CG36 × P26; Choice Genetics, West Des Moines, IA) were individually housed in an environmentally controlled room at the University of Georgia Large

Animal Research Unit (**LARU**; Athens, GA). Barrows were housed in individual 5 m × 1.5 m pens with ¾ slatted and ¼ solid concrete floors. Each pen was equipped with a 2-hole dry feeder (Farmweild, Teatopolis, IL) and nipple waterer to allow for *ad libitum* access to feed and water. For all periods, barrows arrived to LARU prior to supplementation for an acclimation period (25 ± 11 d). Weights were taken every 7 d of acclimation period to calculate ADG. For periods 1 to 3, twenty-four h prior to 10-d supplementation, barrows were weighed, ranked from heaviest to lightest, and randomly allocated to 1 of 2 harvest groups within each 2 barrow strata with group 1 beginning supplementation and group 2 beginning supplementation 4 d later to account for 4 days separation between performance/ harvest days. Within each performance/ harvest group, barrows were ranked by weight and randomly assigned to a NR treatment within each 5- barrow strata. For periods 4 and 5, barrows were weighed prior to arrival at LARU and separated into heavy and light groups. Group 1 arrived a week prior to group 2, barrows were weighed and within each performance/ harvest group, barrows were ranked by weight and randomly assigned to a NR treatment within each 5- barrow strata.

Dietary treatments include a conventional swine finishing diet containing 0 (**CON**) as the negative control group, 15 (**15 NR**), 30 (**30 NR**), 45 (**45 NR**) mg•kg body weight$^{-1}$•d$^{-1}$ NR (Chromadex; Los Angeles, CA), and 0 NR with barrows being supplemented daily 45 mg/kg NR in Karo Syrup (**DRE**; Karo; Chicago, Illinois) for periods 1 to 3 and raw cookie dough (Nestle; Arlington, VA), for easier method of administration, for periods 4 and 5 as the positive control group. Feed weights were consistently recorded each time new feed was added and at d 0 of supplementation, feed remaining in feeders was weighed in order to calculate ADI which was used to formulate NR dosages. At d 5 of supplementation remaining feed was weighed and used to calculate ADI between d 0 and 5. Barrows were re-weighed at d 5 of supplementation and

used in calculation along with d 5 ADI in reformation of NR dosages. Remaining feed was weighed at d 10 of supplementation, 8 to 12 hr prior to the endurance test, to calculate d 10 and overall ADI. This experiment was repeated 5 times; 3 periods with 20 barrows and 2 periods with 19 barrows with 1 barrow dying during final two periods from causes unrelated to supplementation.

***Muscle Biopsy***

On d 0, 5, and 10 of NR supplementation, the right *biceps femoris* (**BF**), *tensor fascia latae* (**TFL**), and *semitendinosus* (**ST**) muscles were biopsied for NAD+ analysis. Biopsy locations were identified with permanent marker and sterilized by removing any hair using clippers (Wahl Professional, Sterling, IL) and debris from the area by aseptically scrubbing the area with concentrated betadine and applying final spray of 70% ethanol. One milliliter Lidocaine with 1% epinephrine was injected subcutaneously into each marked location. After a 10-min wait period, a 6-gauge piercing needle (Precision Needles, Denver, PA) was used to create muscle tissue access for Mammotome elite biopsy gun entry (Mammotome, Cincinnati, OH). Approximately 600 to 700 mg of tissue were collected from each muscle location, immediately placed in 2-ml centrifuge tubes, and submerged in liquid nitrogen for immediate freezing. Biopsy sites were immediately cleaned with 70% ethanol, 20% betadine solution, and Alusheild (Neogen Ideal Animal Health, Lansing, MI) was applied to prevent infection. After collection from all barrows, samples were immediately stored at -80°C until further processing. For d-5 collection, the same biopsy procedure was utilized; however, location was moved approximately 2.54-cm ventral to d 0 biopsy site. Day 10 collection followed same procedure with new location approximately 2.54-cm ventral to d 5 biopsy site.

***Performance Test***

On supplementation d 11, barrows were assigned to a test order and individually subjected to a performance test. Prior to testing, barrows were shaved using hair clippers (Wahl Professional) on the areas covering the left BF, TFL, and ST. Shaved areas were cleaned with soapy water, air dried, and wireless electromyography (**EMG**) electrodes (Noraxon, Scottsdale, AZ) were affixed to each muscle parallel to muscle fiber orientation utilizing KT tape (Hampton-Adams KT Athletic Tape) and livestock glue (Kamar Adhesive, Zionsville, IN) to secure electrodes in place. After EMG application, barrows were walked down a Gait4 pressure mat (GAIT4Dog walkway, CIR Systems Inc., Sparta, NJ) for pre-exhaustion analysis.

For periods 1 to 3, barrows were walked back and forth down a hallway with length of 16.5-m by 2 handlers until subjective exhaustion was achieved. Periods 4 and 5 walked barrows around track with circumference of 55-m. Subjective exhaustion was determined by 5 stops of the barrow, not due to discomfort or distraction, which resulted in handler pressure application to rump. Time was recorded for total exhaustion time and each turn at end of the hallway. Average speed was calculated as distance traveled divided by time run with an average speed of 1.09 m/s. After test, each barrow was walked down Gait4 pressure mat for post-exhaustion analysis. Barrows were loaded and immediately transported 90-m to the University of Georgia Meat Science and Technology Center (**MSTC**; Athens, GA) for harvest.

***Gait4 Mat Analysis***

Prior and immediately following performance test, barrows were walked on a portable walkway system with an active area of 6.10 m in length and 0.61 m in width following the methods of Wang et al. (2021) with slight modifications. Two pre- (**PRE**) and post- (**PO**) performance runs passes down the mat were obtained per barrow. Data evaluated included velocity, cadence, step time, step length, cycle time, and stride length. Velocity was obtained by

dividing distance traveled by ambulation time and is expressed as cm / sec. Cadence was calculated as steps taken divided by ambulation time on the mat translated into steps / min. Stride length as the distance between successive ground contact of the left forelimb expressed in cm. Cycle time as the amount of time for a full stride cycle expressed in sec. Step length was measured on the horizontal axis of the walkway from the landing of the current footfall to the landing of the previous footfall on the opposite foot expressed as cm. Stride length, expressed in cm, was calculated as the distance between the foot landing of two consecutive footfalls of the same foot (right to right, left to left). Averages of all parameters from 2 PRE and 2 PO walkway passes were calculated and recorded.

***Electromyography Analysis***

The methods of Noel at al. (2016) were followed with slight modifications for EMG analysis. Using a custom program in Noraxon MR 3.16 (Noraxon USA, Scottsdale, AZ), raw EMG were processed for each electrical burst corresponding with a muscle contraction by manually applying a 20-500 Hz bandpass filter with high-pass filter as the $6^{th}$ order Bessel filter and low-pass as a $128^{th}$ order FIR filter. The EMG amplitude characteristics were derived as root mean square (**RMS**). Data was individually normalized to first 15 s of each barrow's run and averaged every 15 s during the performance test of each barrow. Each 15 s average of each barrow was reported and utilized for statistical analysis.

***Serum Analyses***

On supplementation d 10, prior to biopsy, barrows were restrained and 12 mL of blood was collected from the jugular vein into red top vacutainer tube (BD Vacutainer, Franklin Lakes, NJ) for PRE-sample analysis. After transportation to MTSC and stunning, barrows were exsanguinated and blood was collected in 12 mL red top blood collection tubes for post-fatigue

(**PO**) sample. Within 10 minutes of collection, samples were centrifuged at 1,115 ×*g* for 10 min at 20°C. Serum was transferred to 2-mL microcentrifuge tubes, placed on ice, and ultimately stored at -80°C until further analyses.

Serum samples were submitted to Clinical Pathology Lab (College of Veterinary Medicine, University of Georgia, Athens, GA) for analyses of cortisol, lactate, creatine kinase (**CK**), and glucose levels PRE and PO. Cortisol was analyzed using Immulite 2000 System Analyzer (Malvern, PA). Creatine kinase, lactate, and glucose were analyzed using Cobas C 31/501 Analyzer (Indianapolis, IN).

***Harvest, Sample Collection, Immunohistochemistry, and Histology***

All barrows were harvested using USDA approved methodology. After chilling for 24 h postmortem, 2.54-cm cores were taken from left BF, TFL, and ST from each carcass at the approximate EMG location for muscle fiber type and succinate dehydrogenase (**SDH**) staining.

A 1-$cm^3$ portion of each core was embedded in optimal cutting temperature (**OCT**, Neg-50, Epredia, Kalamazoo, MI) embedding media, frozen with liquid nitrogen cooled isopentane, and stored at -80°C until further analysis. Two slides with 2 5-µm thick cryosections per slide, 0.5 mm apart, for each muscle sample were collected on positively charge slides (Cardinal Health, Waukegan, IL) for both fiber type and SDH analysis.

The methods of Paulk et al. (2014) were followed for fiber type immunohistochemistry with slight modifications. Cryosections were incubated with blocking solution containing 5% horse serum and 0.2% TritonX-100 in phosphate buffered saline (**PBS**). Cryosections were incubated overnight at 4°C with a primary antibody solution containing blocking solution and 1:10 supernatant myosin heavy chain, type I, IgG2b (BAD5; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, Iowa), 1:100 supernatant myosin heavy chain, type IIB,

IgM (BF-F3; Developmental Studies Hybridoma Bank), and 1:10 supernatant myosin heavy chain, type IIA, IgG1 (SC-71; Developmental Studies Hybridoma Bank). Following incubation, cryosections were washed 3 times with PBS for 5 min. After washing, samples were incubated for 45 min with a secondary antibody solution consisting of blocking solution and 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, 1:1,000 Alexa Fluor 633 goat anti-mouse IgG2b (Invitrogen) for BAD5, 1:1,000 wheat germ antigen Alexa Fluor 594. After secondary incubation, cryosections were washed in PBS 3 times for 5 min. After washing, 5 µL of 9:1 glycerol in PBS was placed on cryosections and were cover slipped for imaging.

Slides with cryosections for SDH staining were incubated at 37°C for 45 min in incubation solution containing 50% nitro blue tetrazolium solution (1% nitro blue tetrazolium in Mili-Q [**MQ**] water), 25% phosphate buffer solution (20% potassium phosphate monohydrate, and 11% disodium hydrogen phosphate in MQ water), and 25% sodium succinate solution (2.7% sodium succinate dibasic hexahydrate in MQ water). After incubation, slides were washed in MQ water, covered in 9:1 glycerol PBS, and cover slipped for imaging.

Four representative photomicrographs were captured per sample at 4× magnification for immunohistochemistry and 10× magnification for SDH staining using a Revolve 4 Upright, Inverted, Brightfield, Fluorescent Microscope (ECHO Laboratories; Radnor, PA). Immunohistochemistry results were obtained by measuring each individual muscle fiber CSA found within WGA fluoresced borders, taking the average of all CSA per muscle fiber type per image and averaging all 4 images per sample. Muscle fiber types were determined based off color displayed under fluorescence where Type IIB fluoresced green, Type IIA fluoresced red, Type IIX was an overlap of green and red fluorescence, and Type I fibers were shown in orange.

Muscle fiber type proportions were calculated by taking the sum of all muscle fibers per image and dividing the number of each muscle fiber type total per image and averaging between the 4 images taken per sample. Data for SDH staining were obtained by measuring each individual fiber type (confirmed by the corresponding immunohistochemical stain image) pixilation using ImageJ software (ImageJ.JS 1.53). Averages of each muscle fiber type between the 4 images per sample taken were calculated and reported.

***Statistics***

Animal feed performance, body weight, and performance test data were analyzed as randomized complete block design with barrow 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 but sorted by muscle. Blood metabolite and gait data were analyzed as a randomized complete block design with repeated measures. 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. Electromyography data were analyzed as a randomized complete block design with a 5 × 3 factorial arrangement and repeated measures. Treatment, Muscle, Time, and their 2- and 3-way 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. All data were analyzed using SAS 9.3 (SAS Institute Inc., Cary, NC). Pairwise comparisons between the least square means of the factor level comparisons were computed using the PDIFF option of the LSMEANS statement. Statistical significance was determined at $P \leq 0.05$ while tendencies were determined at $0.05 > P \leq 0.10$.

## RESULTS

There were no Treatment effects for initial and final BW, pre-supplementation and supplementation d 5 ADG, ADI, and G:F BW ($P > 0.44$; Table 2). There was no Treatment effects ($P = 0.37$) for supplementation d 10 ADG; however, there was a Treatment effect ($P = 0.05$) for ADI and tendency ($P = 0.07$) for G:F between treatments as 45NR had decreased ADI and G:F compared to both CON and 15 NR ($P < 0.05$), which did not differ from each other or all other treatments ($P > 0.28$); however, 45 NR did not differ compared to 30 NR or DRE ($P > 0.09$).

There were no Treatment effects for average speed, time to exhaustion, or distance to exhaustion ($P > 0.17$; Table 3). There were no Treatment × Time interactions or Treatment effects for serum cortisol and glucose ($P > 0.30$). There was no time effect ($P = 0.94$) for serum glucose; however, PO serum cortisol was greater ($P < 0.05$) than PRE.

There were no Treatment effects for fiber type percentage or all fiber type's CSA within BF and ST muscles ($P > 0.20$; Table 4). Within the TFL, Treatment did not affect the type I, or IIB fiber percentages ($P > 0.83$); however, there was a treatment effect ($P = 0.03$) within type IIX fiber and tendency ($P = 0.09$) within type IIA fiber percentage. For both type IIA and IIX there was no difference between CON, 15 NR, 30 NR, and DRE fiber type proportions ($P > 0.15$). Treatment 45 NR did not differ ($P = 0.07$) from DRE but was greater than all other treatment groups ($P < 0.03$). There was no SDH treatment differences within muscle fiber types for BF and ST muscles ($P > 0.63$; Table 5). Treatments did not differ in SDH intensity within type I, IIX, and IIB fibers in TFL muscles ($P > 0.11$), but there was a tendency ($P = 0.08$) within type IIA fibers where 30NR had greater ($P = 0.01$) SDH intensity compared to 45NR but did not differ between all other treatments ($P > 0.09$). All other treatment groups, CON, 15NR, and DRE did not differ from 45 NR ($P > 0.06$), nor each other ($P > 0.20$).

There was no Treatment × Time interaction for serum CK and lactate ($P > 0.30$; Fig 1). There was a treatment effect in both serum CK and lactate ($P < 0.03$; Fig 1a,c), where CON barrows had greater CK and lactate values compared to all other treatment groups ($P < 0.03$), which did not differ from each other ($P > 0.49$). Creatine kinase PO -exhaustion values were greater ($P = 0.04$; Fig 1b) compared to PRE- exhaustion CK values; however, there was no difference ($P = 0.24$; Fig 1d) in time points for serum lactate.

For gait mat analysis, there was no Treatment × Time interaction or treatment effect for velocity, step time, cycle time, cadence, step length, or step length ($P > 0.30$; Fig 2). There was no difference ($P = 0.67$) between time points for step length; however, there were differences seen between time points for all other parameters ($P < 0.05$). Velocity, cadence, and stride length was greater PRE- exhaustion compared to PO- exhaustion ($P < 0.05$), but step time and cycle time both decreased PO- exhaustion ($P < 0.05$).

Electromyography displayed no Treatment × Time × Muscle interaction, Muscle × Time interaction, or Treatment effect ($P > 0.26$; Fig 3). There was a Treatment × Muscle interaction ($P < 0.01$) where treatments did not differ ($P > 0.20$) within ST muscles; however there were Treatment differences within the BF and TFL ($P < 0.01$). Within the BF, CON, 15NR, and 45NR did not differ in RMS values ($P > 0.07$) but had decreased RMS when compared to 30NR and DRE treatments ($P < 0.05$), which did not differ from each other ($P = 0.56$). The TFL demonstrated lesser RMS in CON, 15NR, 30NR, and 45NR groups, which did not differ from each other ($P > 0.15$) compared to DRE ($P < 0.01$); however, 15NR and 30NR treatments, did not differ from DRE ($P > 0.20$ ) which had greatest RMS data. There was a Muscle effect where ST had greatest RMS reported and was greater than both BF and TFL muscles ($P < 0.01$), which did differ ($P = 0.02$) from each other.

There was a Time effect ($P = 0.01$) for RMS. At 0 s, RMS was greater than 105, 120, and 180 s ($P < 0.03$), but did not differ from all other time points ($P > 0.10$). Time 15 s was greater than all remaining time points after and including 75 s ($P < 0.05$), but did not differ from 30, 45, and 60 s ($P > 0.13$), which did not differ from each other ($P > 0.15$). Root mean square at 45 s was not different from all time points ($P > 0.07$) except 105 and 180 s ($P < 0.05$) which had decreased RMS but did not differ ($P = 0.89$) from each other. Values were not different than all other time points at 60 s ($P > 0.08$). Times 135, 150, and 165 did not differ from all other time points ($P > 0.08$), except for 15 s which did differ ($P > 0.04$).

There was also a Time × Treatment tendency ($P = 0.07$) where RMS did not differ between treatments at 0, 90, 105, 150, 165, and 180 s ($P > 0.06$), but at both 15 and 120 s, CON and 45NR barrows had decreased RMS output compared to DRE barrows ($P < 0.01$), but did not differ from 15NR and 30NR barrows ($P > 0.06$), which did not differ from each other at both times ($P > 0.36$). While DRE had the greatest RMS, it also did not differ from 15NR and 30NR ($P > 0.08$). By 30 s, CON RMS decreased ($P = 0.03$) from DRE barrows, but did not differ from remaining treatments ($P > 0.14$), which did not differ from each other ($P > 0.33$). At 45 s, 30NR had greater RMS compared to CON and 45NR treatments ($P < 0.05$), but did not differ from 15NR and DRE ($P > 0.08$), which did not differ from each other or CON and 45NR ($P > 0.38$). Again, at 60 s, 30NR had greatest RMS and differed from CON and 15NR ($P < 0.03$), which did not differ from each other ($P = 0.84$), but did not differ from all other treatments ($P > 0.12$). At 75 s, 30NR was greater than CON ($P = 0.04$), but both CON and 30NR did not differ from all remaining treatments ($P > 0.09$). By 135 s, DRE RMS increased and was greater numerically than all other treatment groups, but only differed statistically from CON and 45NR ($P < 0.03$), which did not differ from each other or 15NR and 30NR barrows ($P > 0.22$).

## DISCUSSION

Pigs arriving to the abattoir non-deceased, non-ambulatory, and non-injured are a growing economic issue in the pork industry (Ritter et al., 2009; Ritter et al., 2020). Limited research has focused on fatigued pig syndrome, especially prevention through dietary supplementation. Nicotinamide adenine dinucleotide, a muscle mitochondria coenzyme, might be key in improving fatigue resistance through its role in ATP production (Li and Suave, 2015). Numerous studies documented mice and human NR supplementation positively affected NAD+ concentration and endurance (Canto et al., 2012; Zhang et al., 2016; Airhart et al., 2017; Conze et al., 2019). Pig dietary NR supplementation has yet to be examined and thus, in the current study, five NR doses were evaluated for their ability to affect NAD+ production, resistance to fatigue, and fiber type oxidative capacity.

During pre-supplementation and initial 5-d supplementation periods, pigs fed NR did not differ in ADI, G:F, or ADG. During the final 5 d of supplementation. 45NR pigs consumed 15% less feed than pigs in other treatments, which resulted in 45NR pigs having 56% greater G:F compared to CON and 15NR pigs. This increase could be attributed to an improvement in growth performance through increased NR supplementation without the need for increased feed intake by the animal. With feed contributing 70% or more to pig production costs, improvement in G:F ratio could provide producers with another incentive to supplement NR. Commonly NR is supplemented in pill form to humans because the product possesses a strong bitter taste. Research on pig tongue attributes indicates a significantly greater number of taste buds in pigs when compared to any other species (Hellenkant and Danilova, 2001); therefore, decreased feed consumption may be attributed to the bitter and unappealing taste of NR, which does not occur until supplemented greater than 30 mg•kg body weight$^{-1}$•d$^{-1}$ which means barrows did not intake

the proper NR-calculated dose. This may dampen maximum effects seen in past-studies which saw signifcant effects when supplementing at dosages greater than 100 mg•kg body weight$^{-1}$•d$^{-1}$ (Canto et al., 2012; Zhang et al, 2016; Conze et al., 2019; Airhart et al., 2017).

Resistance in mice was not reported in other literature, but the increase in endurance was similar in NR supplemented mice (Canto et al., 2012; Zhang et al, 2016). In both past studies, mice supplemented 400 mg•kg body weight$^{-1}$•d$^{-1}$ had increased run time and run duration than their control diet counterparts. In the current study. there was no statistical difference in running distance and time between treatments. Two reasons were identified for this result. First, the negative Treatment effect on feed intake is concerning because supplementation was specifically designed to provide calculated doses based on intake. In looking at the NR dose response shape, it appeared feed intake decreased as NR inclusion level increased and became statistically different at the 45 mg•kg body weight$^{-1}$•d$^{-1}$ rate. Throughout all five repetitions, 20, 25, and 45% of 15NR, 30NR, and 45NR pigs did not meet intake expectations. Therefore, reduced intake most likely eliminated ability of NR barrows to produce more NAD+, which caused no performance measure differences.

The second reason performance data did not yield results as expected may be due to data including all 98 barrows subjected to the experiment. The study was designed to have 10% of the barrows subjected to performance testing removed. Some barrows refused to move and fought most, if not most of the performance run, which led to variation in run duration and time between treatments. Additionally, barrows flagged as “problem runners” were exclusively NR supplemented. Further processing of performance data to remove poor movement barrows may help reduce variation and become a better reflection of the effect of NR on fatigue.

Because of the preliminary nature of data analysis, results are encouraging because DRE barrows numerically ran 16 and 19% father and longer, respectively, compared to other treatment averages. This could be due to direct supplementation and consistency drench feeding offered compared to feed supplementation. Especially considering DRE barrows moved numerically longer and farther than 45NR barrows. Overall, improving feed dosing and developing better muscle fatigue measures may demonstrate the effect of NR on barrow fatigue.

There are previous studies which utilize EMG technology in monitoring fatigue; however, there is yet to be studies utilizing EMG in NR supplemented barrows. Electromyography sensors were attached to ST, TFL, and BF muscles, all of which are greatly important t ambulation in pigs. The current study evaluated RMS response for every 15 s of movement for each barrow during performance tests for the initial 180 s following PRE gait analysis as to analyze all pigs for the same amount of time which was the shortest run period. Root mean square, defined as muscle load assessment in time domain on the basis of amplitude (Bartuzi et al., 2007), serves as a means of evaluating muscle fiber recruitment by comparing active muscle fibers to the number utilized during initial movement (Noel et al., 2016). In regards to muscle fatigue, as subjects' fatigue RMS will increase indicating fiber recruitment followed by a decrease signify fiber exhaustion (Kingugasa et al., 2004; Racinais et al., 2007).

In the current study, overall, there was a decrease in RMS with rises in RMS at 15, 45, and 165 s indicating fiber recruitment. On average, ST had greater RMS values than BF and TFL. This increase seen in ST might be explained through ST muscles utilizing more muscle fibers throughout the run compared to other 2 muscles. In ST muscles there was no NR effect on RMS activity; however, there were 46% increased RMS values in TFL within 30 NR and DRE barrows compared to other treatments and 39% increase RMS in BF muscle of DRE barrows

compared to 45 NR and CON groups, which may indicate increased NR doses could improve overall muscle fiber recruitment abilities. The greater RMS output for TFL and BF muscles, similarly to Noel et al. (2016) study which showed RAC treated barrows had a 26% increase in TFL RMS compared to ST, occurred in order to maintain movement during the performance test. The decreased RMS for 45NR barrows compared to DRE could be attributed to 45NR barrows' decreased feed intake, while DRE barrows were directly administered the product.

Many studies document effects exercise and stress elicit on multiple species' blood metabolites. In humans, equine, and cattle, serum lactate increased as work load and stress increased (Bangsbo et al., 2007; Nozaki et al., 2009; Hagemaier et al., 2017; Daden et al., 2019). Cortisol increased during elevated activity and stress situations due to signaling from sympathetic nervous system's fight-or-flight response. Studies with humans, cattle, and sheep, demonstrated increased cortisol response when subjects were exposed to physical work or stressful situations (Echternkamp, 1984; Nozaki et al., 2009; Cockram et al., 2012). Creatine kinase, as a response to muscle breakage, increased similar to lactate and cortisol after exercise. In past literature, sheep, equine, cattle, and humans all demonstrated increased serum CK after exposure to some form of physical activity (Nozaki et al., 2009; Cockram et al., 2012; Hagemaier et al., 2017; Daden et al., 2019). Much research observing serum glucose and glycogen found an inverse relationship between them. In rats, after muscle contraction stimulation, glucose uptake increased while circulating glucose concentrations decreased (Hespel and Richter, 1990; Jensen et al., 1997).

In the current study, there was no statistical significance seen in glucose results pre- and post-exhaustion; however, numerically, treatments CON and 15NR demonstrated decreased glucose, translating to an increase in glucose uptake to account for muscle stimulation and

energy depletion, while 30NR, 45NR, and DRE barrows had increased glucose concentrations. This numerical increase in greater NR dosed barrows may indicate NR ability to compensate for energy depletion and prevent glucose uptake compared to lesser NR dosage treatments. Cortisol measures in the current study agreed with past literature, where after physical activity concentrations increased; however, no differences were noted between NR doses, indicating NR would not affect the sympathetic nervous system and its response systems during times of stress. Serum lactate levels in the current study, contradictory to past studies, had no change between pre- and post- fatigue. During pre-fatigue blood collection barrows resisted snaring by pulling back on the snare and thus contracting their muscles increasing the production of lactate prior to fatigue. Although lactate did not differ between pre- and post- fatigue, a 35% decrease in lactate levels was experienced in all barrows supplemented NR compared to CON barrows. This treatment difference would lead conclusions of NR being beneficial in decreasing overall lactate levels, which may assist in reduction of pale, soft and exudative pork in the industry (Cobanovic et al., 2020). Similar to previous literature, CK increased 33% due to performance testing and decreased 40% over both time periods in barrows supplemented with NR compared to CON barrows. These results suggest NR assists with energy production to meet the demands for activity in order to decrease need for CK production. Past literature evaluating alternative NAD+ precursors, niacin and tryptophan, on CK and lactate production showed no effect on CK within humans, rats, dairy cattle, or pigs but did show decrease in lactate levels within rats and fish and broiler chickens (Peeters et al., 2004; Guyton and Bays, 2007; Tejpal et al., 2007; Green et al., 2012; Wang et al., 2014; Guo et al., 2017). The NR effects seen in the current study compared to past studies evaluating NR alternatives indicates more potential for NR to assist in creatine kinase and lactate release, both of which are notable metabolites secreted in times of fatigue.

Although gait mat analysis showed no NR effect, all parameters, except stride length, showed differences pre- and post-fatigue. Previous studies comparing fatigued to non-fatigued human gait profiles showed decreased in stride length and cadence and increases in step and cycle time in fatigued subjects (Montes et al., 2011; Kalron, 2015; Ibrahim et al., 2020). Similarly, in the current study, pre- to post-fatigue showed a 20, 12, and 10% decrease in velocity, cadence, and stride length, respectively, and 17 and 14% increase in step time and cycle time. These noticeable differences between the two time points proves the potential usefulness of gait mat analyses in production settings to flag fatigued pigs. Human therapists are introducing gait mat usage into rehabilitation programs following treatment to evaluate efficacy of treatments (Ibrahim et al., 2020). As being done in human medical facilities, the inclusion of gait mats at farms and abattoirs, may increase fatigued pig awareness. When pigs are flagged as possibly fatigued, they may be allowed an increased lairage period or shorter distance to holding pens in order to minimize transport loss due to fatigued pigs.

Over all muscles and barrows, there was 13% Type I, 23% Type IIA, 12% Type IIX, and 52% Type IIB muscle fibers. These distributions were very similar to those reported by Noel et al. (2016) in pig ST, TFL, triceps brachii lateral head, and deltoideus. There were no treatment effects on BF or ST fiber type distribution; however, within TFL there were greater proportions of Type IIX fibers in 45 NR barrows compared to CON, 15 NR, and 30 NR barrows. Although it is difficult to prove, these findings may indicate a transition to more mitochondrial dense Type IIA fibers. Possibly a longer supplementation period would allow for full transition of Type IIX fibers to either IIB or IIA to establish a more clear effect NR may have on fiber types as muscle fibers take at least 2 weeks, and possibly longer, to fully turnover (Plotkin et al., 2021). Although difficult to prove, the current study 45 NR barrows tended to have a greater number in Type IIA

fibers which would indicate a shift towards a more oxidative fiber type. As seen in Noel et al. (2016), the current study, although not statistically analyzed, showed increased CSA going from Type I to Type IIB this is expected as past literature has established an inverse relationship between oxidative capacity and CSA (van Wessel et al., 2010). Though, there was no response noted between treatments for CSA. The current study is the first to examine the NR effect on SDH intensity within barrow muscle cells. Succinate dehydrogenase is found in the mitochondria inner membrane within the electron transport chain, where ATP production is reliant on oxidative phosphorylation (Kern et al., 1999). A more oxidative Type I fiber will have more SDH expressed as decreased intensity than a more glycolytic in nature Type IIB fiber which will be expressed as greater intensity. There was no change between treatments in intensity recording; however, again similarly to Noel et al. (2016) there was increasing in intensity from Type 1 to Type IIB.

**CONCLUSION**

Feeding barrows a formulated diet containing varying dosages of NR for 10 d did not affect endurance capabilities during performance testing; however, through direct supplementation of the greatest dose DRE barrows were capable of running numerically longer and farther than other treatment groups. Electromyography analysis indicated this could be attributed to increased muscle fiber recruitment within BF and TFL muscles. This increase in RMS was not comparable to histology results as there was no difference among treatments and muscles. Overall, EMG technology can be a valuable tool in directly measuring pigs muscle fatigue in real time although not efficient in abattoir settings; however, gait mat inclusion at both farm and abattoir needs to be further studied to determine the usefulness in flagging fatigued pigs and thus reducing transport loss association with them. Future research should also be performed

with extending the period of supplementation time to allow for complete absorption of NR into metabolic system as during the current study's short supplementation period there was an increase in transitional fibers types in conjunction with a tendency to shift to more oxidative fiber type which with increased supplementation time may result in full transition to oxidative fiber types to improve endurance capabilities.

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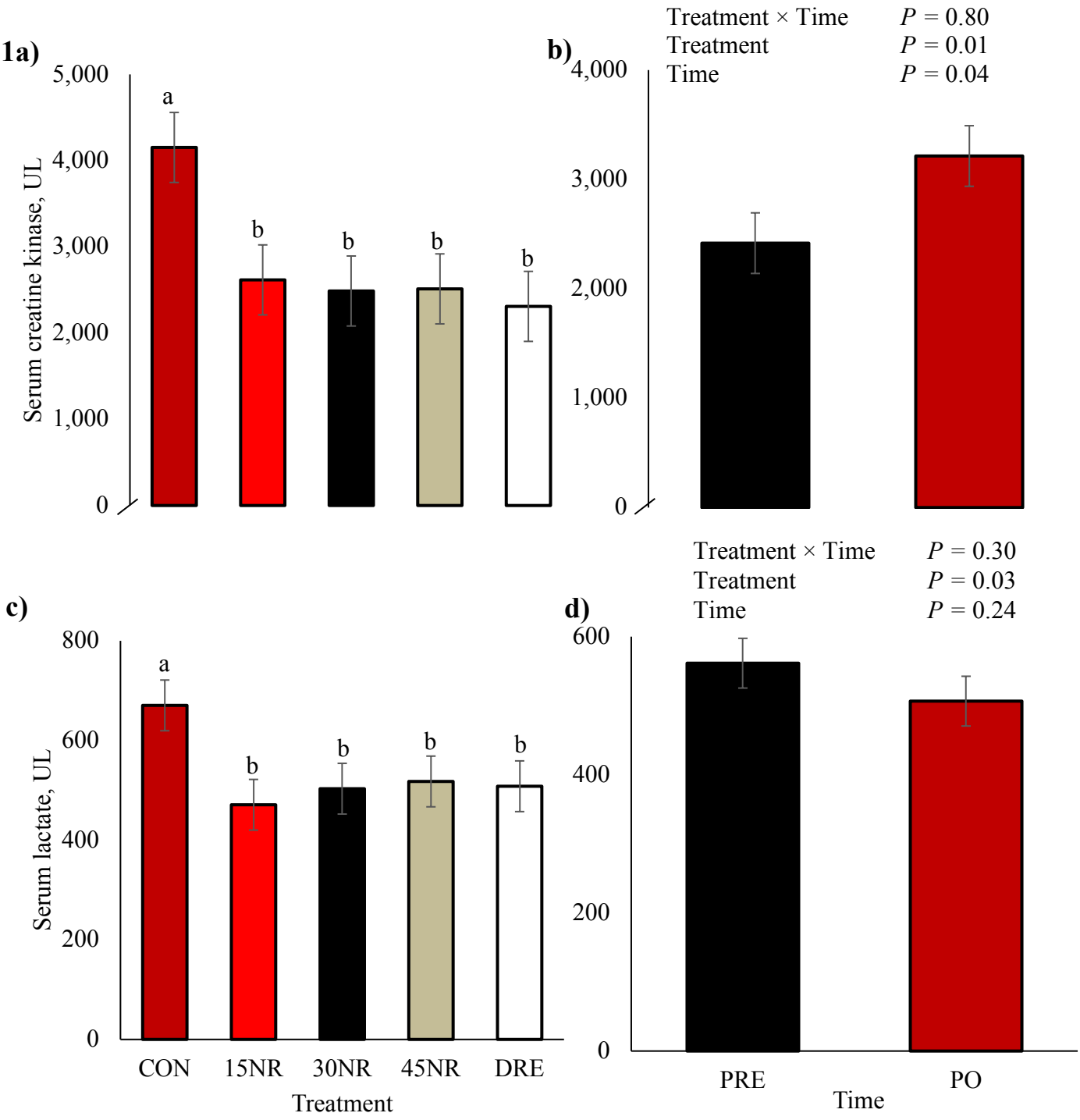
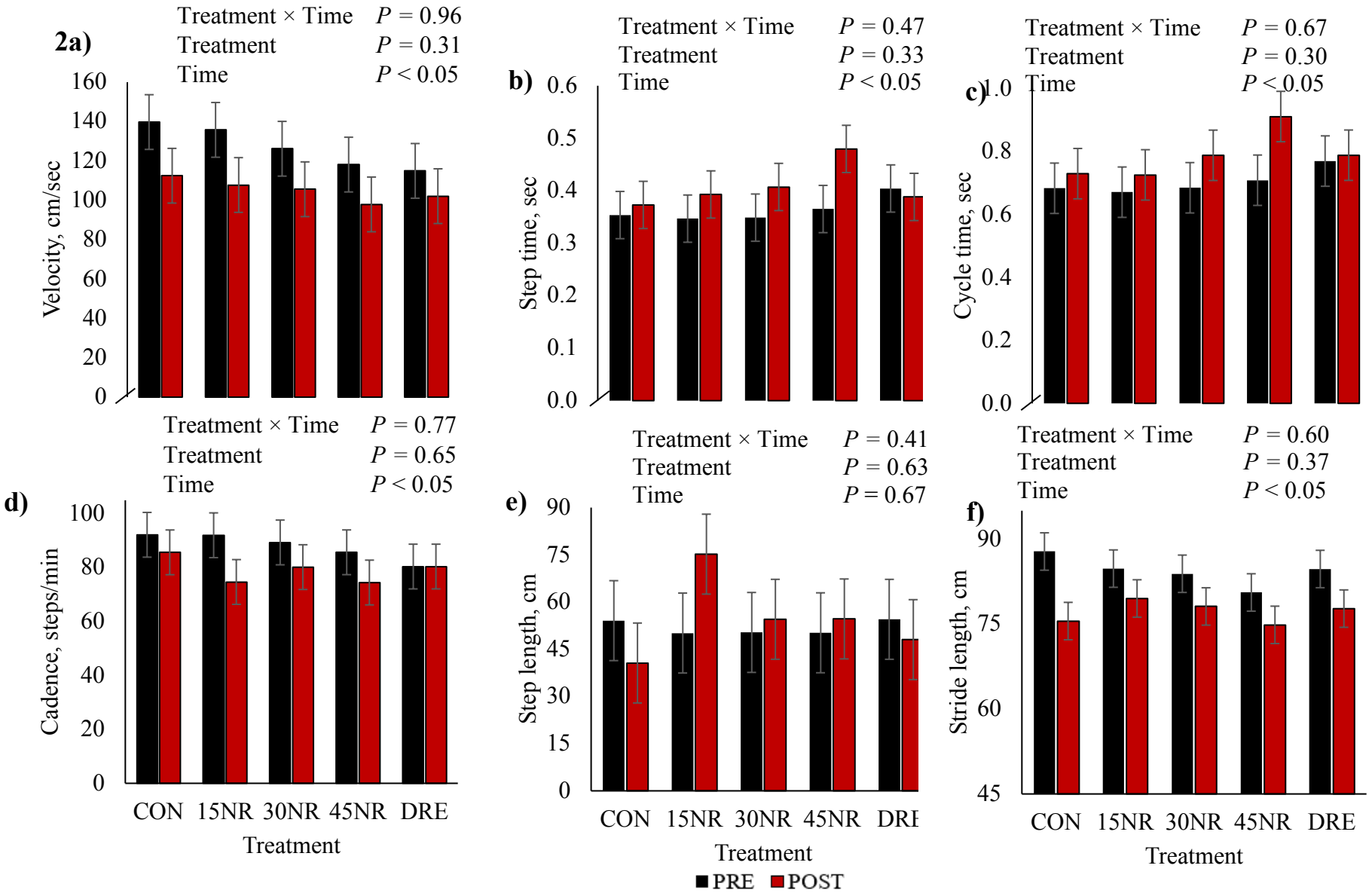
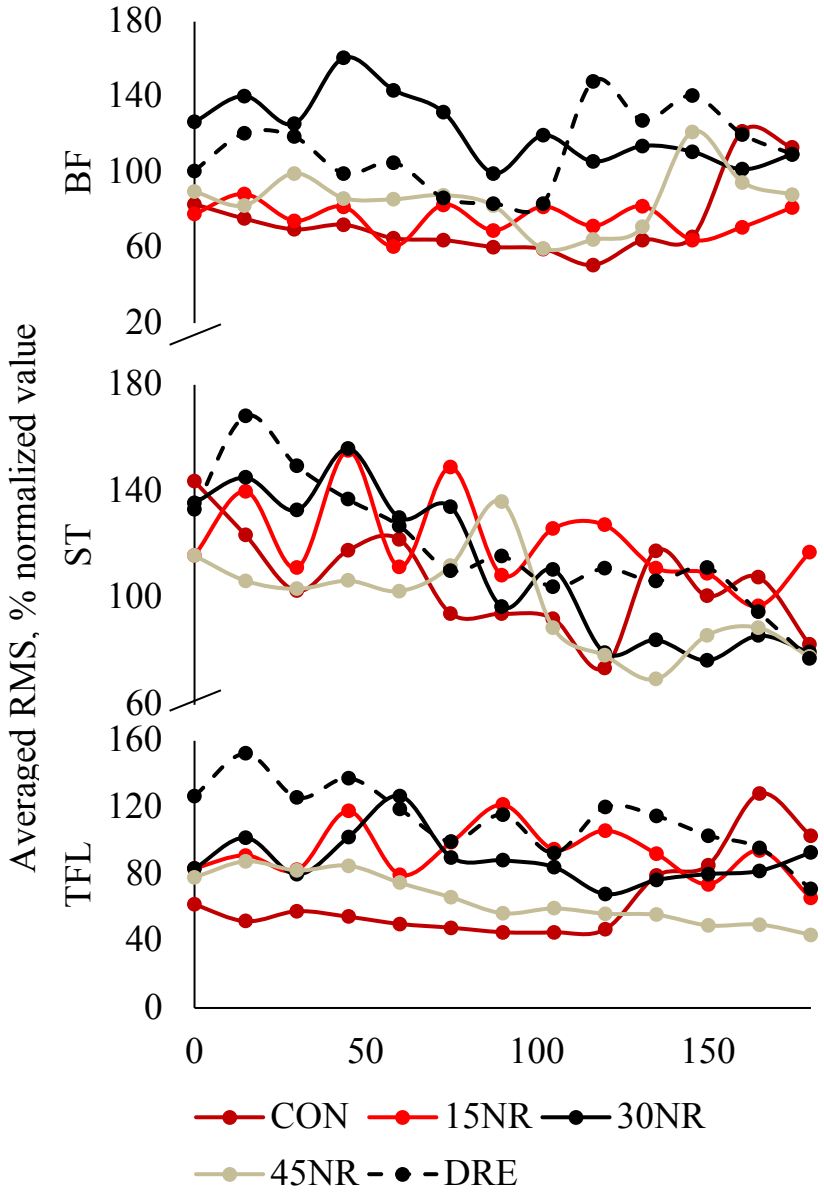

1a)
Serum creatine kinase, UL
5,000
4,000
3,000
2,000
1,000
0
a
b
b
b
b
b)
Treatment × Time P = 0.80
Treatment P = 0.01
Time P = 0.04
4,000
3,000
2,000
1,000
0
c)
Serum lactate, UL
800
600
400
200
0
a
b
b
b
b
CON
15NR
30NR
45NR
DRE
Treatment
d)
Treatment × Time P = 0.30
Treatment P = 0.03
Time P = 0.24
600
400
200
0
PRE
PO
Time

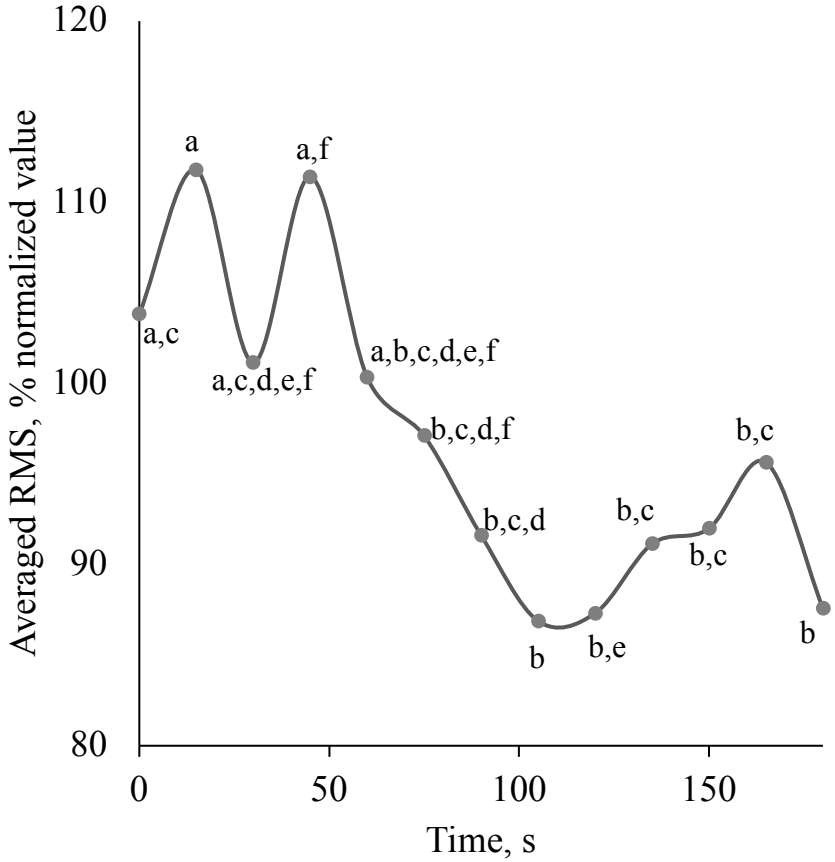

2a)
Treatment × Time P = 0.96
Treatment P = 0.31
Time P < 0.05
Velocity, cm/sec
160
140
120
100
80
60
40
20
0
b)
Treatment × Time P = 0.47
Treatment P = 0.33
Time P < 0.05
Step time, sec
0.6
0.5
0.4
0.3
0.2
0.1
0.0
c)
Treatment × Time P = 0.67
Treatment P = 0.30
Time P < 0.05
Cycle time, sec
1.0
0.8
0.6
0.4
0.2
0.0
d)
Treatment × Time P = 0.77
Treatment P = 0.65
Time P < 0.05
Cadence, steps/min
100
80
60
40
20
0
e)
Treatment × Time P = 0.41
Treatment P = 0.63
Time P = 0.67
Step length, cm
90
75
60
45
30
15
0
f)
Treatment × Time P = 0.60
Treatment P = 0.37
Time P < 0.05
Stride length, cm
90
75
60
45
CON 15NR 30NR 45NR DRE
Treatment
PRE POST

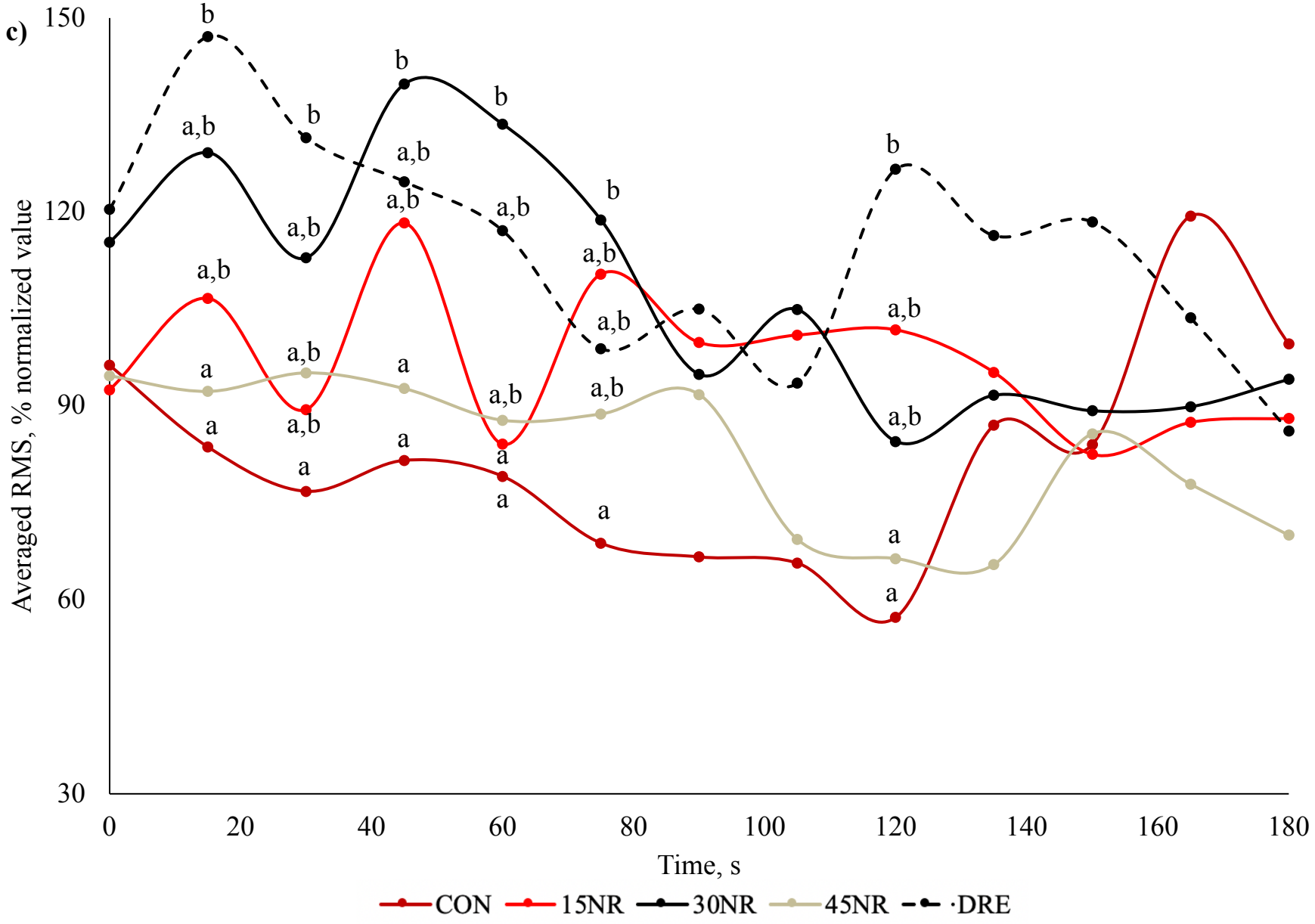
| | |
|---|---|
| Treatment × Time × Muscle | $P = 0.99$ |
| Treatment × Time | $P = 0.07$ |
| Treatment × Muscle | $P < 0.01$ |
| Time × Muscle | $P = 0.50$ |
| Treatment | $P = 0.26$ |
| Time | $P < 0.01$ |
| Muscle | $P < 0.01$ |

**3a)**

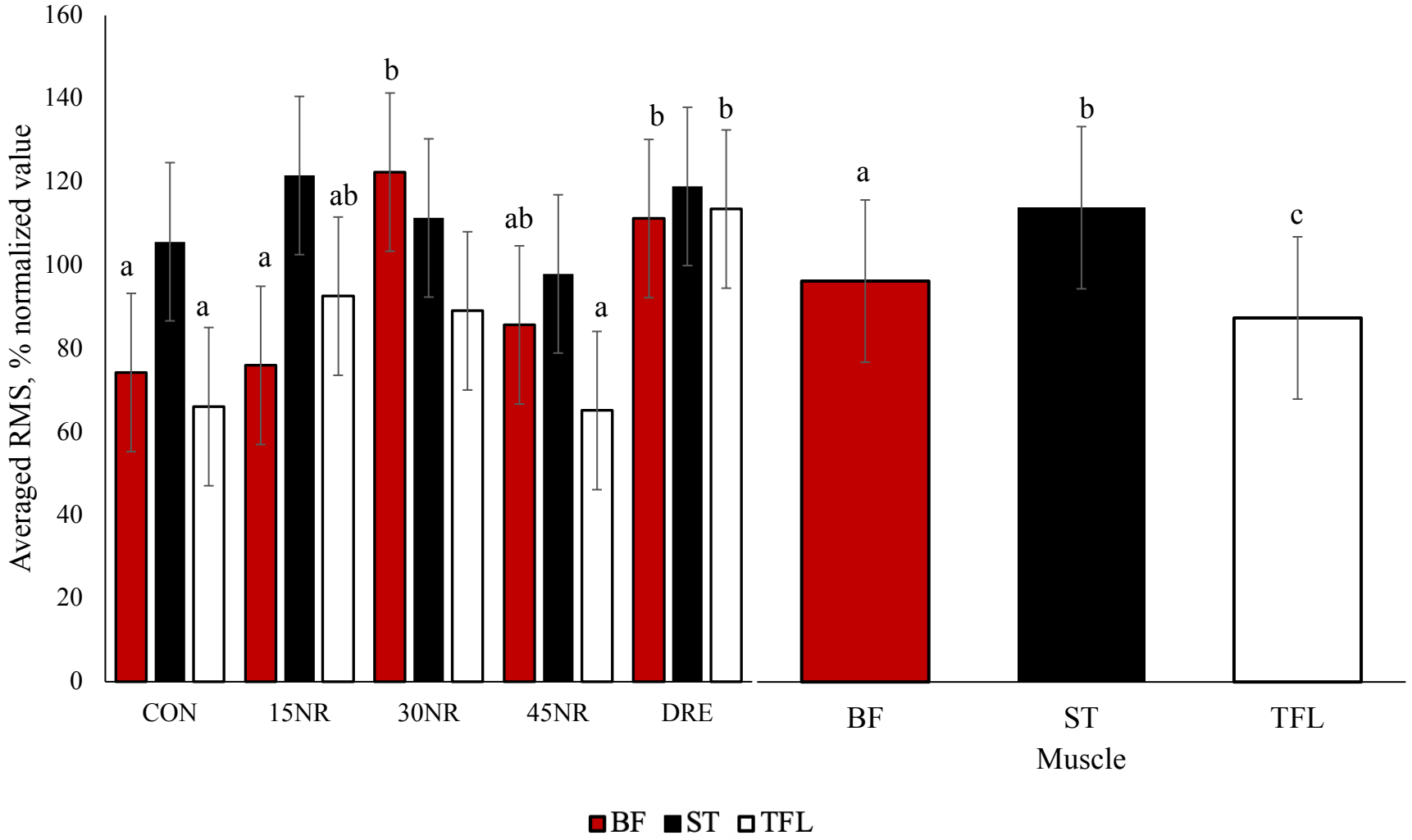



**b)**




c)
150
120
90
60
30
Averaged RMS, % normalized value
0
20
40
60
80
100
120
140
160
180
Time, s
CON
15NR
30NR
45NR
·DRE

**d)** **e)**


160
140
120
100
80
60
40
20
0
Averaged RMS, % normalized value
a
a
a
ab
b
ab
a
b
b
CON
15NR
30NR
45NR
DRE
a
b
c
BF
ST
TFL
Muscle
BF ST TFL

## FIGURES

**Figure 1**. Serum creatine kinase a) treatment and b) time and serum lactate c) treatment and d) time of 98 pigs pre- and post- performance test subjection following 10 d of feeding a conventional swine finishing diet containing 0 (**CON**), 15 (**15 NR**), 30 (**30 NR**), 45 (**45 NR**) mg • kg body weight$^{-1}$ • d$^{-1}$ NR (Chromadex; Los Angeles, CA), and 0 NR with barrows being supplemented daily 45 mg/kg NR in Karo Syrup (**DRE**; Karo; Chicago, Illinois) for periods 1 to 3 and raw cookie dough (Nestle; Arlington, VA) for periods 4 and 5. Barrows were individually walked at 1.09 m/s until subjective exhaustion occurred. Exhaustion was determined as the barrow resisting movement and resisting human application of pressure to rump 5 times. If barrows went down on front limbs, exhaustion was also designated. $^{ab}$denotes mean tend to differ within muscle ($P < 0.05$).

**Figure 2.** Gait4 Mat averaged a) velocity and b) step time c) cycle time d) cadence e) step length and f) stride length of 98 pigs pre- and post- performance test subjection following 10 d of feeding a conventional swine finishing diet containing 0 (**CON**), 15 (**15 NR**), 30 (**30 NR**), 45 (**45 NR**) mg • kg body weight$^{-1}$ • d$^{-1}$ NR (Chromadex; Los Angeles, CA), and 0 NR with barrows being supplemented daily 45 mg/kg NR in Karo Syrup (**DRE**; Karo; Chicago, Illinois) for periods 1 to 3 and raw cookie dough (Nestle; Arlington, VA) for periods 4 and 5. Barrows were individually walked at 1.09 m/s until subjective exhaustion occurred. Exhaustion was determined as the barrow resisting movement and resisting human application of pressure to rump 5 times. If barrows went down on front limbs, exhaustion was also designated.

**Figure 3.** Electromyography (**EMG**) averaged a) biceps femoris (**BF**), semitendinosus (**ST**), and tensor fasciae latae (**TFL**) Time b) Time c) Treatment × Time d) Treatment × Muscle and e) Muscle root mean square (**RMS**) of 40 barrows subjected to performance test following 10 d feeding a conventional swine finishing diet containing 0 (**CON**), 15 (**15 NR**), 30 (**30 NR**), 45 (**45 NR**) mg • kg body weight$^{-1}$ • d$^{-1}$ NR (Chromadex; Los Angeles, CA), and 0 NR with barrows being supplemented daily 45 mg/kg NR in Karo Syrup (**DRE**; Karo; Chicago, Illinois) for periods 1 to 3 and raw cookie dough (Nestle; Arlington, VA) for periods 4 and 5. Barrows were individually walked at 1.09 m/s until subjective exhaustion occurred. Exhaustion was determined as the barrow resisting movement and resisting human application of pressure to rump 5 times. If barrows went down on front limbs, exhaustion was also designated. Data were averaged every 15 s. The RMS values were normalized to initial 15 s walking data. $^{ab}$denotes mean tend to differ within muscle ($P < 0.05$). SEM= 19.0.

**Table 1**. Diet composition (*ad libitum* basis)

| Periods 1 and 2 | | Periods 3, 4, and 5 | |
|---|---|---|---|
| Ingredient, % | | Ingredient, % | |
| Corn | 79.19 | Corn | 79.43 |
| Soybean meal | 16.2 | Soybean meal | 16.2 |
| Fat | 2.0 | Fat | 2.0 |
| Salt | 0.35 | Salt | 0.35 |
| Dicalcium phosphate | 0.42 | Dicalcium phosphate | 0.58 |
| Lysine | 0.24 | Lysine | 0.24 |
| Vitamin[1] | 0.25 | Swine pre-mix[3] | 0.50 |
| Minerals[2] | 0.15 | | |
| Limestone | 1.29 | | |
| Phytase | 0.12 | | |

[1] Vitamin pre-mix provided…

[2] Mineral pre-mix provided…

[3] Swine vitamin and mineral pre-mix provided 3.6% zinc, 3.5% iron, 1.0% magnesium, 3,200 ppm copper, 500 ppm iodine, 60 ppm selenium, 1,000,000 IU/lb vitamin A, 150,000 IU/lb vitamin D3, 4,000 IU/lb vitamin E, and 3 mg/lb vitamin B12.

**Table 2**. Pre-supplementation and supplementation d 5 and d 10 body weights and feed performance of barrows fed a conventional swine finishing diet containing 0 (**CON**), 15 (**15NR**), 30 (**30NR**) 45 (**45NR**) mg • kg body weight$^{-1}$ • d$^{-1}$ and 45 mg/kg NR daily oral drench (**DRE**[1]) nicotinamide riboside

| | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | CON | 15NR | 30NR | 45NR | DRE | SEM | *P*-value |
| Finishing performance | | | | | | | |
| Initial BW, kg | 95 | 94 | 95 | 95 | 96 | 5.1 | 0.96 |
| Final BW, kg | 134 | 133 | 134 | 133 | 133 | 2.9 | 0.99 |
| Pre-supplementation[2] | | | | | | | |
| ADG, kg | 1.01 | 1.07 | 1.01 | 0.99 | 0.95 | 0.07 | 0.60 |
| ADI, kg | 3.56 | 3.48 | 3.49 | 3.38 | 3.51 | 0.22 | 0.72 |
| G:F | 0.29 | 0.33 | 0.30 | 0.31 | 0.28 | 0.03 | 0.51 |
| D5 supplementation[3] | | | | | | | |
| ADG, kg | 1.65 | 1.44 | 1.41 | 1.55 | 1.53 | 0.16 | 0.44 |
| ADI, kg | 3.64 | 3.41 | 3.56 | 3.52 | 3.57 | 0.23 | 0.74 |
| G:F | 0.46 | 0.46 | 0.41 | 0.46 | 0.45 | 0.06 | 0.93 |
| D10 supplementation[4] | | | | | | | |
| ADG, kg | 2.89 | 2.41 | 2.69 | 2.51 | 2.24 | 0.31 | 0.37 |
| ADI, kg | 3.74$^{a}$ | 3.68$^{a}$ | 3.50$^{a}$ | 3.14$^{b}$ | 3.56$^{a}$ | 0.20 | 0.05 |
| G:F | 0.25$^{a}$ | 0.30$^{a}$ | 0.34$^{ab}$ | 0.43$^{b}$ | 0.29$^{ab}$ | 0.05 | 0.07 |

[1] Nicotinamide riboside administered in Karo Syrup (Karo; Chicago, Illinois) for periods 1 to 3 and raw cookie dough (Nestle; Arlington, VA) for periods 4 and 5.

[2] Performance parameters for barrows prior to NR supplementation.

[3] Performance parameters for barrows during first 5 d of supplementation.

[4] Performance parameters for barrows during final 5 d of supplementation.

**Table 3**. Performance test and blood parameters of barrows pre- and post- exhaustion fed a conventional swine finishing diet containing 0 (**CON**), 15 (**15NR**), 30 (**30NR**) 45 (**45NR**) mg • kg body weight$^{-1}$ • d$^{-1}$ and 45 mg/kg NR daily oral drench (**DRE**[1]) nicotinamide riboside

| | Treatment | | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | CON | 15NR | 30NR | 45NR | DRE | SEM | Treatment | Time | Treatment × time |
| Performance test[2] | | | | | | | | | |
| Average speed, m/s | 1.07 | 1.06 | 1.15 | 1.08 | 1.07 | 0.08 | 0.70 | - | - |
| Time to exhaustion, s | 457 | 479 | 441 | 458 | 545 | 39.7 | 0.17 | - | - |
| Distance to exhaustion, m | 477 | 498 | 485 | 500 | 567 | 56.7 | 0.54 | - | - |
| Blood parameter | | | | | | | | | |
| Serum cortisol, µg/dl | | | | | | | 0.85 | <0.01 | 0.99 |
| Pre[2] | 3.62 | 3.50 | 3.91 | 2.69 | 3.58 | 0.91 | | | |
| Post[3] | 14.17 | 13.54 | 13.88 | 13.33 | 13.49 | 0.86 | | | |
| Serum glucose, mg/dl | | | | | | | 0.30 | 0.94 | 0.32 |
| Pre | 86.98 | 84.89 | 87.22 | 86.93 | 86.83 | 5.51 | | | |
| Post | 77.60 | 77.75 | 93.05 | 90.21 | 93.16 | 5.06 | | | |

[1] Nicotinamide riboside administered in Karo Syrup (Karo; Chicago, Illinois) for periods 1 to 3 and raw cookie dough (Nestle; Arlington, VA) for periods 4 and 5.

[2] Barrows were individually walked at 1.09 m/s until subjective exhaustion occurred. Exhaustion was determined as the barrow resisting movement and resisting human application of pressure to rump 5 times. If barrows went down on front limbs, exhaustion was also designated.

[3] Blood was collected on d 10 of supplementation prior to performance test.

[4] Blood was collected immediately following stunning after performance test.

**Table 4.** Immunohistochemistry muscle fiber characteristics of three muscles in barrows fed a conventional swine finishing diet containing 0 (**CON**), 15 (**15NR**), 30 (**30NR**) 45 (**45NR**) mg • kg body weight$^{-1}$ • d$^{-1}$ and 45 mg/kg NR daily oral drench (**DRE**[1]) nicotinamide riboside

| | Treatment[1] | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | CON | 15NR | 30NR | 45NR | DRE | SEM | *P*-value |
| *Biceps femoris* | | | | | | | |
| Fiber type, % | | | | | | | |
| Type I | 0.15 | 0.14 | 0.17 | 0.14 | 0.14 | 0.01 | 0.36 |
| Type IIA | 0.22 | 0.27 | 0.26 | 0.27 | 0.24 | 0.02 | 0.58 |
| Type IIX | 0.11 | 0.08 | 0.07 | 0.08 | 0.09 | 0.02 | 0.66 |
| Type IIB | 0.52 | 0.51 | 0.49 | 0.50 | 0.53 | 0.02 | 0.83 |
| CSA[2], μm$^2$ | | | | | | | |
| Type I | 2,753 | 3,049 | 3,019 | 3,102 | 3,112 | 299 | 0.90 |
| Type IIA | 3,917 | 4,392 | 4,440 | 4,575 | 4,146 | 393 | 0.78 |
| Type IIX | 5,148 | 5,476 | 6,335 | 5,894 | 5,674 | 558 | 0.64 |
| Type IIB | 5,775 | 6,524 | 6,662 | 6,258 | 6,260 | 624 | 0.70 |
| *Semitendinosus* | | | | | | | |
| Fiber type, % | | | | | | | |
| Type I | 0.07 | 0.08 | 0.08 | 0.07 | 0.12 | 0.02 | 0.22 |
| Type IIA | 0.20 | 0.19 | 0.15 | 0.24 | 0.24 | 0.03 | 0.29 |
| Type IIX | 0.16 | 0.13 | 0.16 | 0.16 | 0.11 | 0.03 | 0.46 |
| Type IIB | 0.57 | 0.60 | 0.61 | 0.53 | 0.53 | 0.03 | 0.35 |
| CSA, μm$^2$ | | | | | | | |
| Type I | 3,583 | 4,433 | 4,252 | 4,507 | 3,603 | 515 | 0.47 |
| Type IIA | 4,338 | 4,796 | 4,980 | 6,098 | 4,668 | 606 | 0.20 |
| Type IIX | 5,264 | 6,238 | 6,231 | 6,840 | 6,589 | 759 | 0.55 |
| Type IIB | 4,589 | 5,801 | 5,463 | 5,997 | 6,260 | 805 | 0.46 |
| *Tensor facia latae* | | | | | | | |
| Fiber type, % | | | | | | | |
| Type I | 0.14 | 0.16 | 0.17 | 0.16 | 0.15 | 0.02 | 0.88 |
| Type IIA | 0.27$^{a}$ | 0.26$^{a}$ | 0.27$^{a}$ | 0.20$^{b}$ | 0.22$^{ab}$ | 0.03 | 0.09 |
| Type IIX | 0.12$^{a}$ | 0.09$^{a}$ | 0.11$^{a}$ | 0.18$^{b}$ | 0.13$^{ab}$ | 0.02 | 0.03 |
| Type IIB | 0.52 | 0.51 | 0.49 | 0.50 | 0.53 | 0.02 | 0.83 |
| CSA, μm$^2$ | | | | | | | |
| Type I | 1,879 | 1,698 | 2,059 | 1,849 | 2,085 | 249 | 0.74 |
| Type IIA | 2,324 | 2,455 | 3,243 | 2,388 | 2,753 | 341 | 0.31 |
| Type IIX | 3,263 | 3,450 | 4,282 | 3,459 | 3,839 | 409 | 0.43 |
| Type IIB | 3,890 | 3,912 | 4,661 | 4,241 | 4,367 | 394 | 0.57 |

[1] Nicotinamide riboside administered in Karo Syrup (Karo; Chicago, Illinois) for periods 1 to 3 and raw cookie dough (Nestle; Arlington, VA) for periods 4 and 5.
[2] CSA = cross-sectional area

**Table 5.** Succinate dehydrogenase muscle fiber characteristics of three muscles in barrows fed a conventional swine finishing diet containing 0 (**CON**), 15 (**15NR**), 30 (**30NR**) 45 (**45NR**) mg • kg body weight$^{-1}$ • d$^{-1}$ and 45 mg/kg NR daily oral drench (**DRE**[1]) nicotinamide riboside

| | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| Item | CON | 15NR | 30NR | 45NR | DRE | SEM | *P*-value |
| *Biceps femoris* SDH Intensity[2], AU | | | | | | | |
| Type I | 120 | 122 | 135 | 123 | 127 | 17.8 | 0.63 |
| Type IIA | 144 | 146 | 150 | 146 | 148 | 14.8 | 0.97 |
| Type IIX | 162 | 167 | 165 | 170 | 160 | 13.2 | 0.84 |
| Type IIB | 181 | 186 | 185 | 186 | 177 | 11.7 | 0.89 |
| *Semitendinosus* SDH Intensity, AU | | | | | | | |
| Type I | 133 | 131 | 130 | 131 | 135 | 16.9 | 0.99 |
| Type IIA | 148 | 140 | 146 | 151 | 147 | 14.5 | 0.90 |
| Type IIX | 158 | 159 | 157 | 154 | 160 | 14.9 | 0.99 |
| Type IIB | 181 | 178 | 174 | 181 | 181 | 12.4 | 0.95 |
| *Tensor facia latae* SDH Intensity, AU | | | | | | | |
| Type I | 121 | 110 | 108 | 92 | 119 | 16.9 | 0.29 |
| Type IIA | 143$^{ab}$ | 129$^{ab}$ | 151$^{a}$ | 118$^{b}$ | 145$^{ab}$ | 13.6 | 0.08 |
| Type IIX | 168 | 146 | 169 | 145 | 161 | 11.1 | 0.11 |
| Type IIB | 188 | 170 | 188 | 165 | 186 | 10.9 | 0.11 |

[1] Nicotinamide riboside administered in Karo Syrup (Karo; Chicago, Illinois) for periods 1 to 3 and raw cookie dough (Nestle; Arlington, VA) for periods 4 and 5.
[2] SDH = succinate dehydrogenase; 0 = most intense staining and 250 = less intense staining; AU = arbitrary units

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